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Semi-Mechanistic Pharmacokinetic and Pharmacodynamic Modelling of a Novel Human Recombinant Follicle Stimulating Hormone

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A novel recombinant follicle stimulating hormone (rFSH), FE 999049, expressed in a cell-line of human fetal retinal origin (PER.C6®) is in development at Ferring Pharmaceuticals for controlled ovarian stimulation in infertility treatment to achieve functional oocytes for assisted reproductive technologies. In this PhD, population pharmacokinetic (PK) and pharmacodynamic (PD) modelling with nonlinear mixed effects models was used to analyse the PK and PD properties of FE 999049.

The first PK model based on first-in-human single dose data with FE 999049 was a one-compartment model with a delayed absorption described by a transit compartment. A similar model was then used to describe the repeated dose pharmacokinetics of FE999049 observed after multiple dosing in a second phase I study. The model differed by having an endogenous FSH contribution to the total measured FSH concentration. Furthermore, progesterone baseline levels had an inhibitory feedback effect on the estimated endogenous FSH baseline value. Over time the inhibin B levels further suppressed the endogenous FSH production. In a semi-mechanistic PKPD model, the rFSH, endogenous FSH, and inhibin B concentrations were modelled simultaneously with total FSH stimulating the inhibin B production rate, and inhibin B levels inhibiting the endogenous FSH production.

Body weight was a covariate explaining some of the variation in apparent clearance and apparent volume of distribution in all models. Through simulations it was found that having body weight as a covariate at the PK parameters resulted in an overall decrease in drug exposure and inhibin B response with increasing body weight.

In this work it was furthermore identified that endogenous FSH levels have to be considered in the model else parameters can become biased and total FSH exposure is under-estimated in the model, especially at baseline. Additionally, this thesis demonstrates it is of importance to account for the hormone dynamics and negative feedback from the ovarian hormones in order to accurately describe the change in endogenous FSH levels over time. The standard method of baseline correcting data might therefore not be adequate as it will result in an overcorrection at later time points since the endogenous level decrease during treatment.

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Dissertation for the degree of
Doctor of Philosophy in applied mathematics

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Preface

With an interest in applied mathematics, in particular modelling within human physiology, pharmacokinetic (PK) and pharmacodynamic (PD) modelling in drug development was the ideal topic for my PhD work. The project was an industrial PhD as a collaboration between the department of Science, Systems, and Models at Roskilde University and Experimental Medicine in Ferring Pharmaceuticals. It was a privilege having both an academic and an industrial affiliation and very interesting working in these two different research environments.

Data from three clinical trials with a recombinant follicle stimulating hormone (rFSH), FE 999049, for use in infertility treatment were provided by Ferring pharmaceuticals for analyses and development of population PK and PKPD models. As modelling is an interdisciplinary field the optimal results are achieved with knowledge from all the fields as well as expertise in software for model implementation and data analysis. This PhD thesis demonstrates how the multiple disciplines are needed for understanding the system of interest and developing mathematical models. Furthermore, the focus is on how these models can be used in acquiring knowledge about an investigational medicinal product in development and the models potential use in simulations and for predicting drug response.

The thesis starts with a general introduction to the fields and the overall background of infertility, the drug development process, and need for modelling and simulation (Chapters 1-2). In the next chapters the more extensive theory behind this work is covered. The underlying physiology for the female reproductive endocrine system is described in Chapter 3 with an outline of the major hormones involved, their interaction, and role in follicular development. Chapter 4 gives a historic perspective of the development in gonadotropin therapy and FSH compounds. The chapter also provides an overview of the important factors influencing the PK and PD properties of FSH compounds as well as markers for ovarian response that have potential in predicting treatment outcome and individualising treatment. This is important knowledge in determining what relations and covariates are relevant to include in the modelling and for dose selection.

General methods for population data analysis and development of nonlinear mixed effects models are presented in Chapter 5. A review of current mathematical models with FSH products is given in Chapter 6, which leads to what is relevant to investigate in this PhD and the specific research objectives in Chapter 7.

Chapter 8 lists the three clinical trials with generated data and analysis methods used in this PhD for development of the PK and PKPD models. The final models and parameters are reported in Chapter 9 along with illustrations of the results and use of the models in simulations. Lastly the thesis is rounded of with a discussion (Chapter 10) and conclusions (Chapter 11) of the findings from this

work and put into perspective of the current available knowledge.

The research resulted in three manuscripts, one for each of the clinical trials, included at the back of the thesis:

Paper I: Population Pharmacokinetic Modelling of FE 999049, a Recombinant Human Follicle Stimulating Hormone, in Healthy Women after Single Ascending Doses
Rose, T.H., Röshammar, D., Erichsen, L., Grundemar, L., and Ottesen, J.T.
(Submitted)

Paper II: Characterisation of Population Pharmacokinetics and Endogenous Follicle Stimulating Hormone (FSH) Levels after Multiple Dosing of a Recombinant Human FSH, FE 999049, in Healthy Women
Rose, T.H., Röshammar, D., Erichsen, L., Grundemar, L., and Ottesen, J.T.
(Submitted)

Paper III: Semi-Mechanistic Pharmacokinetic-Pharmacodynamic Modelling of Inhibin B Levels after Multiple Doses of the Recombinant Human Follicle Stimulating Hormone FE 999049 in Infertile Women
Rose, T.H., Röshammar, D., Karlsson, M.O., Erichsen, L., Grundemar, L., and Ottesen, J.T.

This industrial PhD was financed by Ferring Pharmaceuticals with funding from the Innovation Fund Denmark under their 3-year industrial PhD programme.

Abstract

Infertility is an increasing worldwide problem and it can be devastating for a couple not being able to conceive a child on their own. Controlled administration of recombinant follicle stimulating hormone (rFSH) can be used in infertility treatment to achieve functional oocytes for assisted reproductive technologies. A novel rFSH, FE 999049, expressed in a cell-line of human fetal retinal origin (PER.C6®) is in development at Ferring Pharmaceuticals. Drug development is a long and costly process, it is therefore important to extract and utilise all possible information from clinical trials.

In this PhD, population pharmacokinetic (PK) and pharmacodynamic (PD) modelling of data from three clinical trials with FE 999049 was used to analyse the PK and PD properties of FE 999049. A population approach with nonlinear mixed effects models was used in order to facilitate identification of variation between subjects and causes hereof.

The first PK model based on the first-in-human single dose data with FE 999049 was a one-compartment model with a delayed absorption described by a transit compartment. Body weight was a covariate explaining some of the variation in apparent clearance (CL/F) and apparent volume of distribution (V/F). A similar model was then used to describe the repeated dose pharmacokinetics of FE999049 observed after multiple dosing in a second phase I study. A few adjustments were made from the first model according to differences in study designs. The model differed by having an endogenous FSH contribution to the total measured FSH concentration. Furthermore, feedback from ovarian hormones at endogenous FSH levels were identified and accounted for in the model. Progesterone baseline levels had an inhibitory effect on the estimated endogenous FSH baseline value. Over time the inhibin B levels further suppressed the endogenous FSH production. Body weight was confirmed as a covariate at CL/F and V/F. The interaction between rFSH, endogenous FSH, and inhibin B was further investigated in a semi-mechanistic PKPD model. Modelling the hormone concentrations simultaneously facilitated incorporation of continuous stimulation and feedback over time. The model adequately described the hormone dynamics with total FSH stimulating the inhibin B production rate, and inhibin B levels inhibiting the endogenous FSH production.

Through simulations of different patient and dosing scenarios it was found that having body weight as a covariate at the PK parameters resulted in an overall decrease in drug exposure with increasing body weight. A doubling of weight required almost a doubling of dose to get same exposure. Consequently, with FSH stimulating inhibin B production, the inhibin B response was lower for higher body

weight.

In this work, it was furthermore identified that endogenous FSH levels have to be considered in the model else parameters can become biased and total FSH exposure is under-estimated in the model, especially at baseline. Additionally, this thesis demonstrates it is of importance to account for the hormone dynamics and negative feedback from the ovarian hormones in order to accurately describe the change in endogenous FSH levels over time. The standard method of baseline correcting data might therefore not be adequate as it will result in an overcorrection at later time points since the endogenous level decrease during treatment.

A simultaneous dose-concentration-response model for inhibin B levels can be of value in quantifying and predicting ovarian response in clinical studies and clinical settings since inhibin B is an indicator of follicular growth and the earliest measured ovarian hormone response to FSH treatment.

Dansk Resumé (Danish summary)

Fertiliteten falder med alderen, og med en generel tendens til at kvinder bliver ældre inden de får børn, er der et øget behov for fertilitetsbehandling. Det kan være et hårdt psykisk pres ikke selv at være i stand til at blive gravid naturligt og skulle gennemgå hormonbehandling. Derfor er der behov for nye hormonpræparater med innovative behandlingsforløb tilrettet den enkelte patient.

Ferring Pharmaceuticals udvikler et rekombinant follikelstimulerende hormon (rFSH), FE 999049, fra en human cellelinie (PER.C6®), der skal injiceres subkutant til kvinden for at modne flere æg til brug i reagensglasbefrugtning (IVF) og mikroinsemination (ICSI). I denne PhD analyseres FE 999049's egenskaber og variation i resulterende koncentration og effekt ved hjælp af populations farmakokinetisk (PK) og farmakodynamisk (PD) modellering af data fra tre kliniske forsøg.

Den første model baseret på det første humane datasæt fra enkelt dosering af FE 999049 i raske frivillige var en en-kompartments PK model med en transit-kompartment i absorptionsprocessen for en forsinket optagelse. Det var nødvendigt med nogle få tilpasninger af modellen før den kunne bruges til at beskrive FE 999049 farmakokinetikken efter multiple doser i det andet fase I studie på grund af forskellene i forsøgsdesignet. Dette inkluderede et endogent FSH bidrag til den samlede målte FSH koncentration. Den endogene FSH startværdi inden dosering af FE 999049 var negativt korreleret med progesteron startværdien. Inhibin B hæmmede yderligere FSH produktionen over tid via en negativ feedback loop. Til sidst var en semi-mekanistisk PKPD model udviklet fra data fra et fase II multiple dosis studie for nærmere at undersøge forholdet mellem FE 999049, endogent FSH og inhibin B respons. Hormon koncentrationerne blev modelleret simultant for at muliggøre beskrivelsen af hormondynamikken over tid med FSH, der stimulerer inhibin B produktionen i ovarierne, og inhibin B der hæmmer den endogene FSH produktion i hypofysen.

Gennemgående i de tre modeller var kvindens kropsvægt en statistisk signifikant kovariat på parametrene clearance og fordelingsvolumenet. Modellerne blev brugt til at simulere FSH koncentrationen og inhibin B respons i kvinder med forskellige kropsvægte. Både FSH og inhibin B koncentrationen faldt med stigende kropsvægt.

Resultaterne i denne PhD indikerer, at det er vigtigt at inkludere endogent FSH i modellering og tage højde for den endogene hormondynamik, der forårsager variation i koncentrationen over tid, ellers kan der forekomme bias i parameterestimaterne, og FSH koncentrationen bliver underestimeret. Dette sår derfor tvivl om hvorvidt standardmetoden med baseline korrektion for et endogent substans

er valid, da FSH afviger betydeligt fra baseline værdien over tid.

Inhibin B er en indikator for follikeludviklingen og er den tidligste respons variabel, der er blevet målt efter FSH behandling. Derfor kan PKPD modellen være brugbar i forudsigelsen af ovariernes respons, idet inhibin B bliver prædikteret som en kontinuer variabel over tid simultant med FSH koncentrationen ud fra dosis.

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I am deeply thankful to my supervisors who have made this PhD possible:

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Lars Erichsen for introducing me to the great field of population PKPD modelling and giving me a variety of exciting research possibilities. Thank you for guiding and supporting me and sharing your expert modelling knowledge.

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To my boyfriend Tobias, thank you so much for your patience and support, for always making me smile and being able to cheer me up even after really hard and long working days. I love you.

Abbreviations

AFC	Antral follicle count
AMH	Anti müllerian hormone
ART	Assisted reproductive technologies
BMI	Body mass index
BQL	Below quantification limit
CHO	Chinese hamster ovary
COC	Combined oral contraceptives
CS	Clinical study
EBE	Empirical Bayes estimates
EMA	European Medicines Agency
FDA	U.S. Food and Drug Administration
FO	First order estimation
FOCE	First order conditional estimation
FSH	Follicle stimulating hormone
GnRH	Gonadotropin releasing hormone
HCG	Human chorionic gonadotropin
HMG	Human menopausal gonadotropin
HPG	Human pituitary gonadotropin
IIV	Interindividual variability
i.m.	Intramuscular
i.v.	Intravenous
IVF	In vitro fertilisation
ICSI	Intracytoplasmic sperm injection
IU	International units
LH	Luteinizing hormone
LLOQ	Lower limit of quantification
MBDD	Model-based drug development

M&S	Modelling and simulation
NONMEM	Nonlinear mixed effect modelling
OFV	Objective function value
PD	Pharmacodynamic
PK	Pharmacokinetic
PsN	Pearl speaks NONMEM
rFSH	Recombinant follicle stimulating hormone
RSE	Relative standard error
s.c.	Subcutaneous
SE	Standard error
VPC	Visual predictive check
WHO	World health organisation

Contents

Chapter 1

Introduction

Challenges in drug development are to find a potent compound, establish its efficient and safe dose with a minimum of side effects, while keeping costs down. Regulatory authorities outline guidance documents with recommended approaches for drug development to assist the industry, as well as setting high demands and requirements for every step in the development process. This includes how to find first-in-human safe starting dose [1, 2], guidance on study design, data analysis, and reporting of results [3–5]. To get a new drug approved, substantial evidence for its safety and beneficial effect is thus required. To obtain this, drug development becomes a long, expensive, and cumbersome process. It is therefore essential to optimise the design and interpretation of clinical trials as well as extracting as much information as possible from the resulting data about the safety, tolerability, and effect of a new investigational medicinal product. An important tool for this is mathematical modelling and simulation.

When a drug enters the body it is absorbed, distributed throughout the body, and in the end cleared from the system by metabolism or excretion. These pharmacokinetic (PK) processes determine the drug concentration in the blood, which also is called drug exposure. The drug concentration depends on the dose and varies over time. In addition, variation between patients occur due to person specific factors. This can be anything from body weight, age, and gender to more complicated influences like disease pathology, hormone levels and other intrinsic factors, as well as extrinsic factors such as concomitant use of other drugs, smoking, or food intake. It is crucial that the drug concentration is high enough to give the intended drug effect over time but still without causing severe side effects. The pharmacodynamics of a drug is the effect caused by the drug, both beneficial and adverse. The drug effect or response to the drug is registered using pharmacodynamic (PD) endpoints which can be biological markers in the body, clinical efficacy measures, or clinical outcomes including unwanted side effects. There might be a delay before an effect can be measured or observed if it is the result of receptor binding and intracellular signalling cascades, or if the clinical outcome evolve over time, e.g. reducing tumour size.

Mathematical models can describe and estimate the drug concentration over time (PK profile) for a given dose and identify factors (covariates) influencing the results. In a complete PKPD model the PK model for the concentration-time profile is related to observed effects in form of PD endpoints for a description of

dose-concentration-response and their combined time course. A population approach with nonlinear mixed effects modelling provides specific information about individuals relative to the population mean, types of variability, and opportunity of examine and perhaps quantify potential covariates' influence at the variability. Appropriate models satisfactorily describing the observed data can then be used for simulation to predict patient outcome, estimate the optimal dose for individuals based on the most significant factors, or to evaluate different study designs.

This PhD thesis concerns population PK and PKPD modelling of FE 999049, which is a recombinant follicle stimulating hormone (rFSH) expressed in a cell-line of human fetal retinal origin (PER.C6®). It is under development at Ferring Pharmaceuticals to be used for controlled ovarian stimulation in order to induce multiple follicular development in women undergoing in vitro fertilisation (IVF)/intracytoplasmic sperm injection (ICSI) treatment. This quantitative work aims at further characterising the PK and PD properties of FE 999049 and variation in drug exposure using nonlinear mixed effects modelling. Furthermore, the influence of endogenous hormone levels and covariates will be investigated in order to facilitate simulation and prediction of infertility treatment outcomes.

Chapter 2

Background

Infertility is an increasing health issue and there is a need for better treatments to catch the great diversity in causes and types of infertility. There are some marketed products for infertility treatment but there is still a medical need for new drugs with innovative treatment protocols. To market a new drug it is often desired it should be superior to existing ones or present new opportunities for treatment strategies. Model-based drug development is a useful approach for assessment of a new drug's potential, dose selection, and study design considerations both in internal decision making and in regulatory interactions.

2.1 Infertility

The world health organisation (WHO) has defined primary infertility as the inability to conceive within two years of trying to obtain a pregnancy, and secondary infertility as inability to become pregnant again after having an earlier birth [6]. Clinicians often define infertility as inability to become pregnant after one year of trying [7, 8].

The infertility prevalence varies between countries and regions. In addition, the different definitions and whether all women, married women, or only child-seeking women are used as basis for the calculation, adds to the difference in reported prevalences. Some estimate that 1 out of 10 couples suffers from infertility, which worldwide is equivalent to more than 80 million people [7]. Another article has stated that it is 80 million couples rather that individuals [9]. A large systemic analysis of health surveys from 1990 to 2010 [10] accessed infertility, measured as not having a live birth over a 5-year exposure period. They found that the overall rates in those years were the same but due to population growth the absolute number of infertile couples was 48.5 million in 2010, an increase of 6.5 million since 1990. The secondary infertility increased with age from 2.6% in child-seeking women of age 20-24 years to 27.1% for age 40-44 years, whereas primary infertility had a mean value around 2.5% and decreased slightly with age.

With the clinical definition, 15% of couples are unable to conceive within the first year, constituting in the US alone at least 6 million infertile couples (Review [8]). Based on data from 2198 infertile couples the cause of infertility was due to female ovulation disorders in 17.6% of the cases while 25.6% had unexplained infer-

tility. Treatment options for these infertility types include hormone therapy, since the cause is not ovarian failure and the ovaries are still responsive to stimulation. The other registered infertility factors were tubal disease (23.1%), endometriosis (6.6%), luteal phase, cerivical, and uterine defect (3.2%), and male factors (23.9%). A Dutch study with 726 couples found that 25.9% and 30% had ovulation disorders and unexplained infertility, respectively [11]. In this study 12.9% of the couples had infertility caused by tubal disease, 3.6% by endometriosis, 27.7% by cervical factor, and 30% had male infertility factors. They experienced that the mean age of females visiting an infertility clinic increased from 29.1 years in 1985-1993 to 31.2 years in 2002-2003 [12]. Similar trends were observed at a Dutch fertility clinic [13]. In 1985, 256 women visited the clinic with a mean age of 27.7 years, by 2008 these numbers had increased to 594 women and 31.4 years, respectively. A further indication of an age shift was that the proportion of women $\geq$ 35 years increased from 7.9% to 31.2%. Even though these results are from The Netherlands, the studies refer to that the trend of delaying childbirth is observed in most developed countries, so this should be representative for other western countries.

In developing countries the prevalence is general higher but also with great variations between regions and countries. In a report by WHO [6] it is estimated that overall more than 1 out of 4 married women in developing countries are infertile adding up to 186 million women whereof 18 million had primary infertility. A similar trend of an increase in secondary infertility with age was observed but here reaching 62% of women trying to become pregnant at age 45-49 years. The reproductive age range in this report is 15 to 49 years, thus wider than the others, and is the reason for that the reported number is larger than the worldwide number. The number also include 17% self-reported infecundity from women who have never menstruated or not menstruated for at least 5 years and postmenopausal women. WHO compared the estimate with previous data and opposed to western countries infertility has overall decreased over time. Sexual transmitted diseases could potentially affect infertility but WHO found no trend with extent of HIV. A possible explanation for the decline could be due to the progress within assisted reproductive technologies (ART) and easier accessibility for women to infertility treatment leading to an increase of women seeking help.

Definitions of infertility and thereby prevalence varies but one thing is certain, infertility is a major worldwide problem and for those affected it can become a life crisis with psychological pressure and a great loss. The age of women starting to have children have increased and as fertility decreases with age, the demand for infertility treatment also increases. The cause of infertility varies greatly and up to 30% have unexplained infertility, so one kind of treatment do not fit all. Hormone treatment can be used for ovulation disorders, which accounts for around 20% of the cases, and if personal factors are taken into account for individualising

treatment, success rates may increase. In unexplained infertility where no damage to ovaries are found, individualised hormone treatment could possibly be useful for timing of mature oocytes and maybe diminish the psychological stress factor when getting personal treatment. Hence, hormone therapy can potentially be used in 50% of all infertile cases. A new hormone compound with an individualised specific treatment protocol have thus potential for being marketed.

2.2 Drug Development

Development of a new drug starts with a drug discovery phase where targets and possible therapies are identified through research based on knowledge of the therapeutic area and disease pathology [14–16]. Thousands of compounds are being tested in order to find candidates of therapeutic value. The most promising, could be several hundred, compounds are selected for further preclinical in vitro laboratory tests and in vivo animal testing to assess the drug's safety profile and effect. It also has to be considered if the candidate drug can be developed at a large scale for marketing. At the end only a few compounds enter clinical trials.

In Phase I clinical trials doses are administered to healthy volunteers to determine whether the drug is safe in humans, as well as investigating its pharmacokinetic properties. The potential dose range is examined in single ascending dose studies with sequential groups given gradually increased doses starting from a low dose in the first group. A safe dose can be tested in a multiple dose study for further assurance of safety and effect after repeated administration. Several trials can be performed to test the drug in different population groups. Phase I studies typically include in the order of 20 to 100 subjects [15–17].

Phase II clinical trials continue to evaluate safety but overall focus is on effectiveness in patients, PKPD relationship, and short-term side effects. This phase is often divided into two parts: a smaller phase IIa proof of concept study in about 100 patients to assure the potential of the drug, and a larger phase IIb study (100-500 patients) where an optimal dose is established. If successful in proving an effective dose with acceptable side effects the drug can proceed into phase III. Based on the results and evaluations, a large scale phase III trial in a diverse target population group (thousands of patients) is undertaken to establish significant confirmatory evidence of drug safety, efficacy, and verify optimal dose of the drug in all subpopulations. More than one phase III trial can be required [15–17].

The overall timeline to get regulatory approval for a new drug is 10-15 years. All along the way communication with regulatory authorities, review boards, ethic committees, and local authorities occur for monitoring and approval of trials to ensure all requirements are fulfilled, and most importantly that trial participants are not exposed to unnecessary risks. After final approval and marketing of a drug,

additional studies are carried out with the purpose of continuing documenting beneficial and adverse effects, since when marketed a much larger population is exposed to the drug. A postmarketing phase IV trial can also be conducted to investigate long-term safety or effect in a specific subgroup of patients [15–17].

All these precautions and stepwise evaluations through the trials also aim to kill non-effective and non-safe drugs as early as possible, but some drugs are still terminated late in the development process. To get one drug approved, money and time have thus been spend on thousands of other compounds, and even clinical trials for other candidates that ended up being discarded. There is not one number for what the average amount of money spend in total on research and development is before one new drug is approved. Different cost estimates and trends over the years were listed in a large systemic review [18] of published articles from 1979 to 2010 that gave estimates of drug development costs. They operated with two types of costs: cash and capitalised costs. The cash number is the actual costs spend on research and development for one new drug to reach the market. Capitalised costs include in addition opportunity costs, which is the amount of money that could have been earned from investing the actual cost elsewhere instead of spending them at research and development. Wide variation was observed and an overall increase in costs over the years from 92 to 883.6 million US$ cash with a corresponding capitalised cost estimate of 161 and 1799 million US$, respectively.

The U.S. Food and Drug Administration (FDA) reported in 2004 the number to be between 0.8 and 1.7 billion US$ [14]. In 2009 estimates of drug development and marketing costs for one drug was estimated to be in the range 1.3 - 1.7 billion US$. That is approximately a doubling of the costs since 2003 [19]. It is primarily the costs of clinical trials that are increasing. These ranges might be for capitalized costs if following the above defined numbers, but it is not mentioned what these estimates include.

In the beginning of this century the pharmaceutical industry increased research and development and was successful in marketing many of the new drugs discovered [19]. With several drugs at the market it became harder to develop and prove the benefits of a new drug to those already marketed. Hence larger trials with more patients had to be conducted in order to establish if there is a small improvement in the new drug compared to old drugs.

It also seemed like a maximum for the success rate of drugs that entered clinical investigation was reached. The success rate had increased from 12% for drugs being developed in the 1960s and 70s to 24% for drugs entering clinical trials in the 1990s. It then decreased to 11.7% again in 2010 (Review [18]). The regulatory demands have also been increased causing a longer development process and sometimes requires extra or bigger trials making it harder and more expensive to market a drug. These criteria cannot be circumvented, but a way to lower costs is catching

and stopping non promising drugs as early as possible and before entering more costly late stage development phases [19]. In addition, costs may be reduced by expediting the clinical development program by more efficient use of the generated data and knowledge about the molecule.

The problem with these challenges, inefficiencies, and rising costs in drug development has also been addressed by FDA in 2004 [14]. In the report it is stressed that there is an urgent need for improving the drug development path. Scientific research and collaboration across fields and institutions are essential for building knowledge and finding better tools and technologies for all levels in drug development. As a toolkit for better effectiveness in data management and analysis, FDA proposes model-based drug development (MBDD) as an important approach that potentially can improve the process significantly.

2.2.1 Model-Based Drug Development

It was not without challenges to apply modelling and simulation (M&S) to drug development. For proper implementation was needed software, knowledge, and sufficient quality data with the right measurements of interest. Another challenge was to convince people it was worth using resources and time at this complicated interdisciplinary task, and incorporate it in clinical trial protocols and reports [20]. Population modelling was, in spite of implementation difficulties, reviewed to be of great value in every phase of drug development to obtain important information about PK and PD properties, and provide insight in individual response and subgroups at risk with need for different dosage regimen.

In 1997, Lewis B. Sheiner introduced the concept of learn-confirm cycles to the drug development phases for a more efficient and informative process [21]. Phase I trials are a first informative step for investigating quantitative questions to gather knowledge and learn about the drug's properties. The phase I study designs also fit nicely in the learning scheme with either a wide range of doses in a diverse population group for PK analysis or as a multiple dose study with several endpoints measured to get an initial indication of the pharmacodynamics and toxic effect. What is learnt about safe and tolerable doses should then be confirmed in a phase IIa study. After the first learn-confirm cycle the results are evaluated to see whether it was possible to properly confirm satisfactory efficacy, and on this basis judged if the drug is qualified to proceed in further development. The second learn-confirm cycle consists of phase IIb (dose finding) and phase III/IV (confirm dosage regimens). If the benefit/risk profile is acceptable the drug is eligible for approval. Data from learning trials is analysed by a population modelling approach and used to design the confirmatory trials, where statistical tests are performed with a null hypothesis of no efficient treatment effect of the drug. Software and capable scientists to perform this model-based data analyses

and simulations were in short supply at the time [21]. The paradigm did not present any single new design ideas, but a development framework including a more science oriented focus instead of mostly having a confirmatory motivation for proving efficacy with the goal of approval. The need for model-based analysis to achieve a greater understanding through learning was emphasised.

This paradigm is comprised in MBDD except with a less sharp distinction of learning and confirming phases in two cycles, but rather a continuous view of learning throughout all phases while confirming previous results [22]. The first step in implementing models in drug development was moving from an empirical decision making to a model-aided development process, where models are used only occasionally and primarily in later phases for decision confirmation and supporting labelling [23]. The further transition to MBDD is difficult, but optimally modelling should be applied throughout drug development. Starting in preclinical development for evaluation and identification, and proceeding into translational PKPD modelling for extrapolation and scaling to humans. In the clinical phases modelling should be used for analytical and predictive purposes as well as for post-marketing surveillance. Pharmaceutical companies doing so may be able to safely enter patient studies faster with increased success rate and at a lower cost. Sheiner and Steimer (2000) [24] gave an extensive list of examples of M&S in drug development proving that modelling was gaining impact.

The usefulness of modelling in phase I was not acknowledged initially, therefore an expert meeting was held to evaluate M&S in phase I [25]. Modelling is invaluable in some phase I tasks like handling missing or censored data and describing complex exposure-biomarker relationship. In particular for sparse data, population modelling is the only way to achieve proper information and interpretation of data. Additionally, a population approach with nonlinear mixed effects modelling enable analysis and quantification of variability and covariate effects. Other advantages of mathematical models are they facilitate data integration from different clinical trials, enable clinical trial simulations to test different study designs, predict results, and extrapolate results to future patients, dose selection, and aid in better informed development decisions of go, pause, or stop based on quantitative decision criteria of treatment effect [22, 26]. Physiological-based modelling and mechanistic models that incorporate knowledge of the biological system and physiological parameters are increasingly used. Mechanistic models quantitatively describe (some of) the processes from drug administration to effect, e.g. drug distribution, receptor mechanisms, interactions with endogenous substances, and feedback mechanisms. Hereby increasing prediction accuracy and improve extrapolation properties of the model [24, 27, 28].

For successful implementation of M&S, PKPD modelling specialists, biostatisticians, and clinical research experimentalists have to work closely together with

mutual trust and acceptance of challenges and contributions from each group. It takes time to overcome scientific differences between each group's custom methods and language. Through tight collaboration with clear communication and interactions, an understanding of the benefits of MBDD can be achieved as well as confidence in model-informed decisions and trials. When successful, the models can be used to support registration of the drug, and convince research and development management of M&S is a powerful tool for more efficient drug development [29]. Planning trials properly is very important as with a wrong dose selection, subjects, duration, or time of sample measurements, the results can be insufficient for analysis or conclusion. The trial will then have to be repeated with a different design which is a costly affair. MBDD can thus help avoiding unnecessary extra development costs. MBDD has even been used to skip phase IIa completely by utilising prior knowledge [22]. Using a MBDD approach can also reduce the number of required patients and shorten the study duration [30]. To obtain this, it required a willingness from the company to change, a training program for the involved employees, and support from senior management. The effort was rewarded with increased probability of success in the studies and a yearly cost reduction of $100 million.

Another important factor for extending the use of M&S is to communicate the results in an understandable way not only to the development team and management, but also very importantly to the regulatory authorities. In 1999 FDA issued a guidance document on how and when to use population PK approach and how protocol, data, and reports should be handled [5]. It was followed in 2003 by a guidance on using PKPD modelling in exposure-response analysis [4]. European Medicines Agency (EMA) released a guideline in 2007 for reporting results from population PK analyses [3] and have since had numerous presentations and workshops on M&S. The great usefulness of M&S was acknowledged by regulatory authorities, and hereby establishing M&S as an important tool in drug development. Today this is widely recognised, though still not implemented as a standard tool for every new drug.

Two recent papers [31, 32] evaluate whether the impact of M&S at drug development has been as great as expected. In their perspective to some extend it has, but it can become even greater. The papers point out that a problem is that in some instances it may be difficult to internally convince some companies of the value of a model based approach. A continuing problem is the lack of understanding of the modelling results for the non-modelling-specialist members of the development team. This is due to the modellers lack of presentation skills. It is emphasised that optimal usage of M&S can only be achieved through proper timing and team work from the beginning to ensure that all are on board with the goals and what the purpose of the M&S is. Else the risk is that at the end the

generated results from M&S are hard to believe for the rest of the team. It is also of uttermost importance that the modellers can present the results in line with the goals and in an understandable way. In addition Bonate [32] stress that the true MBDD revolution will not happen until the M&S results are being presented outside the clinical pharmacology field to make it more broadly understood and hereby get greater influence.

Chapter 3

The Female Reproductive Endocrinology

In order to satisfactorily describe the impact of the pharmacological drug therapy in a mechanistically correct hence useful model, it is necessary to understand the underlying physiology and anatomy of the system of interest. A comprehension of the components and drug response factors in the system will also enable identification of relevant PD endpoints. Thus the reproductive endocrinology involving FSH and follicular development will be presented in this chapter. At first the overall structure and components will be introduced, thereafter more details about the hormonal interplay and development steps are described.

3.1 Anatomical and Physiological Overview

The reproductive endocrine system consists of the hypothalamic-pituitary-gonadal axis and involves several hormones from the brain and ovaries. Interactions between the hormones occur at all levels through feedback loops, resulting in complicated dynamics which eventually leads to maturation of an oocyte and timed ovulation. The major hormones involved in the dynamics and control of reproductive function in females are gonadotropin releasing hormone (GnRH) from the hypothalamus, follicle stimulating hormone (FSH) and luteinizing hormone (LH) from the anterior pituitary gland, and inhibin B, progesterone, and estradiol from the ovarian follicles. Many other hormones, neurotransmitters, and additional components are involved in the underlying mechanisms behind the effects but these details are beyond the scope of this chapter. The overall connections in the reproductive system is illustrated in Figure 3.1. The effects of the hormones will be described in the following sections.

The hypothalamus is part of the diencephalon at the base of the brain and plays an essential role in neurohormonal control of the endocrine system by influencing the pituitary function (Review [33, 34]). The pituitary gland, also called hypophysis, is an extension of the hypothalamus consisting of an anterior and a posterior part with distinct functions. Magnocellular neurons in the hypothalamus have long axons that extend to the posterior pituitary where neurohormones are transferred and subsequently released for regulation of homeostasis and stim-

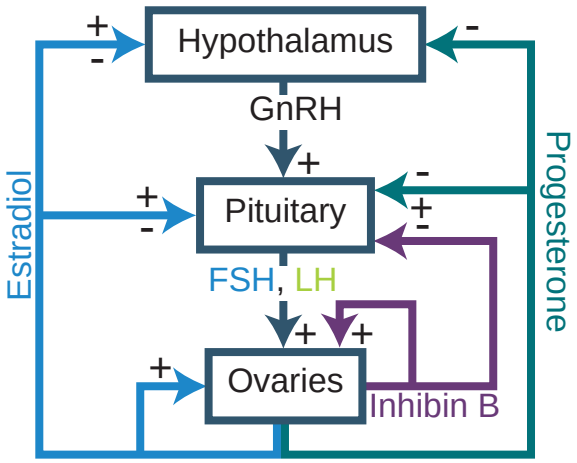

Figure 3.1: A simplified diagram of the hypothalamic-pituitary-gonadal axis in the female with the major hormones and feedback loops. GnRH: gonadotropin releasing hormone, FSH: follicle stimulating hormone, LH: luteinizing hormone. A + indicates a stimulatory effect and a - an inhibitory/supressive effect.

ulation of lactation and uterine contraction. A neural connection does not exist between the anterior pituitary gland and the hypothalamus. Instead, hormones are transported through blood vessels making up the the hypothalamic-hypophyseal portal system. It originates in a capillary bed at the median eminence in the hypothalamus and is connected via long portal veins to a capillary bed in the anterior pituitary. Hypothalamic parvocellular neurons terminates at the median eminence releasing neuropeptide hormones to be taken up by the portal system. These hypophysiotropic hormones affect the anterior pituitary release of hormones involved in growth, metabolism, lactation, stress, sexual differentiation, and reproduction. The pituitary hormones exert their effect at endocrine glands and organs throughout the body.

The principal target organs for pituitary hormones affecting reproduction in females are the ovaries, which contain follicles of different sizes. A growing follicle consists of an oocyte surrounded by layers of granulosa cells and theca cells. The follicles and oocyte develop under tight hormonal control and usually only one follicle per menstrual cycle reach full maturation. At ovulation the mature oocyte is released and transported to the uterus by the fallopian tube. The remaining cell layers of the ovulatory follicle become the corpus luteum, that secretes hormones preparing the uterus for potential implantation of a fertilised egg and exert feedback control to the brain. If the oocyte is not fertilised, menstruation occur and the corpus luteum degenerate.

3.1.1 Reproductive Hormones of the Brain

In reproduction the hypophysiotropic hormone secreted by hypothalamic neurons is the decapeptide GnRH (Review [33, 34]). Upon reaching the anterior pituitary via the portal system GnRH binds to specific GnRH receptors and stimulate the synthesis and release of FSH and LH. GnRH can enhance it's own effect by up-regulation of its receptors at the pituitary. GnRH is secreted in a pulsatile manner with changing frequency during the menstrual cycle in response to integrated neuronal signals and hormonal feedback as well as influence from other substrates. The different frequencies is a way for one hormone to control different release of two hormones from the same gland and even the same cells. In general low GnRH pulse frequencies increase FSH secretion and higher frequencies favours LH secretion, but other hormones also affect the net production and secretion. Another reason for the pulsatile secretion is that the sensitivity of pituitary GnRH receptors decrease if exposed to constant levels of GnRH, and ultimately the pituitary is rendered unresponsive to GnRH. Therefore, controlled GnRH pulses are essential for normal FSH and LH secretion and thus reproductive function, since the major functions of FSH and LH are to induce follicular development and ovulation by stimulating the ovaries.

FSH and LH are gonadotropins belonging to the glycoprotein hormone family that in addition include human chorionic gonadotropin (HCG) and thyroid stimulating hormone (Review [33, 34]). Glycoproteins are heterodimers consisting of two amino acid chains, an α- and β-chain. The α-chain is common to all glycoproteins but the β-chains differ which provide unique specificities, properties, and effects of each hormone. The polypeptide subunits are glycosylated to different extent and there exist various isoforms of each glycoprotein giving rise to altered structure and activity. The higher glycosylation the longer half-life but lower receptor affinity than the more basic isoforms (Review [35, 36]).

FSH and LH are synthesised by gonadotrope cells (gonadotropes) of the anterior pituitary gland. These cells are the only ones that express the genes for the FSH and LH β subunits. An increased synthesis does not necessarily mean increased hormone secretion as they can be stored in secretory vesicles and later released by exocytosis. They are endocrine tropic hormones since they exert their effect after transported in the circulation to the target organ, the ovaries. In the ovary FSH and LH binds to cell surface receptors at follicular cells activating intracellular signalling leading to follicular growth, production of ovarian hormones, oocyte maturation, and ovulation.

The effect of FSH and the necessity of it in maturation of follicles was made clear in 1931 by Fevold et al. [37], but studies investigating reproductive function started even earlier. The first study indicating a role of the pituitary in gonadal function date back to over 100 years ago [38]. In the 1920's Zondek and colleagues

had extensively studied the anterior pituitary and hormones involved in ovarian function [39, 40]. At the same time a different group demonstrated that the anterior pituitary affect sexual maturity (Review [41]). It was generally believed that the anterior pituitary secretes two distinct hormones affecting the ovaries. Fevold et al [37] set out an experiment to prove it and separated the two hormones from the anterior pituitary: a gonad stimulating factor and a luteinizing factor. They established their individual effects in rats. Giving the gonad stimulator on its own resulted in growth of the follicles and was found to be similar to Prolan A - which was the name Zondek had given FSH. The luteinizing hormone could not stimulate the ovaries on its own, but if given after the gonad stimulator hormone such that growing follicles were present, the hormone caused luteinization and creation of corpus luteum. The hormones separated were FSH and LH and their overall effects were thus established in 1931.

FSH is the key hormone in follicular development and growth. It stimulates the granulosa cells to increase FSH receptor expression, it induces LH receptors at the granulosa cells, and increases progesterone, estradiol, and inhibin B production by increasing enzymatic activity (Review [33, 34]). In addition to causing luteinization by a high LH surge and subsequently corpus luteum formation and maintenance, LH stimulates production of estrogens and progesterone by the corpus luteum. Before ovulation, LH stimulates production of progesterone in the theca cells as well as androgens, which are transferred to the granulosa cells for conversion to estrogens under stimulation by FSH. The theca cells cannot perform the final aromatisation of androgens to estrogens, since they lack the enzyme aromatase responsible for the conversion. The granulosa cells, on the other hand, cannot synthesise androgens so these have to be received from the adjacent theca cells. Hence, both LH stimulation of the theca cells and FSH stimulation of the granulosa cells are required for estrogen production. This is the two-cells-two-gonadotropin concept. Later in the follicular development when LH receptors are expressed at granulosa cells due to FSH stimulation, LH acts in synergy with FSH at the granulosa cells too. Just before ovulation LH induce progesterone receptors at the granulosa cells.

3.1.2 Ovarian hormones

The hormones produced by the ovaries in response to gonadotropin stimulation include inhibin B, estradiol, and progesterone (Review [33, 34]). Inhibin B is a peptide hormone consisting of two peptide subunits. In the same peptide family are also inhibin A, activin, and follistatin, which are involved in regulation of FSH levels. The major effect of inhibin B is its important role in inhibiting FSH synthesis and secretion via negative feedback to the pituitary. Furthermore inhibin B can both reduce the number of pituitary GnRH receptors and block the GnRH

induced up-regulation of GnRH receptors. Other effects of inhibin B include enhancing the stimulating effect of LH at the theca cells for increased production of androgens. Inhibin B is produced by the small follicles and is thus an early marker for follicular development [42]. The main source of inhibin B production is the granulosa cells, but it is also produced in other tissues.

Estradiol and progesterone are steroid hormones derived from cholesterol by steroidogenesis. The process consists of different paths of alterations of cholesterol by enzymatic activity. After a few steps, progesterone is produced and can act as a precursor for androgens, which in turn can be transformed to estrogens. Progesterone is secreted by both granulosa and theca cells, whereas estradiol is the main estrogen secreted only by the granulosa cells. They are smaller than peptide hormones and can thus enter the target cells for evoking their effect, which is both paracrine, autocrine, and endocrine through feedback loops.

Progesterone is only produced in small amounts before ovulation but when high levels of estradiol are present, low levels of progesterone are involved in stimulation of LH and FSH secretion for the timing of high surges and ovulation. After ovulation progesterone plays an important role in preparation of the uterus for implantation and in a negative feedback signal at LH and FSH secretion. At the hypothalamic level progesterone suppress the GnRH secretion, and it reduce the pituitary response to GnRH.

Estradiol plays a significant role in escalating ovarian growth and cell proliferation. It acts on granulosa cells in a self-promoting manner by increasing the number of its own as well as LH and FSH receptors and by stimulating aromatase activity. Besides local effect in the ovaries, estradiol is also involved in longer feedback loops for regulation of gonadotropin production and secretion both direct and indirect. Low levels of estradiol have a negative feedback effect at FSH and LH secretion, while high levels stimulate LH secretion. To exerts the positive feedback at LH, highly elevated estradiol levels have to be sustained over a longer period. The high estradiol level is essential in causing the ovulatory LH surge in multiple ways. It amplify the GnRH effect at the pituitary, increase the number of GnRH receptors, and up-regulate the intracellular signalling system in the gonadotropes. Estradiol also induces progesterone receptor expression and hereby enhance the effect of progesterone.

3.2 Follicular Development

Follicular development is a continuing process happening from fetal life until menopause with both gonadotropin independent and dependent stages (Review [33, 34]). Only aproximately 400 out of the two million primordial follicles present at birth will become fully mature and ovulate. A primordial follicle contains an

oocyte at a resting state with a single layer of spindle shaped pre-granulosa cells surrounding it. At all times resting primordial follicles enter the pool of growing follicles independent of gonadotropin stimulation. Likewise, oocytes are lost continuously at any point in the development through atresia by apoptosis which is programmed cell death of the follicular cells. It takes several months for a follicle to reach a fully mature state.

In the first development step from a primordial follicle to a primary follicle the oocyte is slightly enlarged and the surrounding cells proliferate to cuboidal shaped granulosa cells (Figure 3.2). When several layers of granulosa cells are formed the follicle develop into a secondary follicle. Surrounding stroma cells that have acquired its own blood supply form an outer layer of the follicle which differentiate into two theca layers. The theca externa contains smooth muscle and collagen and is involved in contraction causing the rupture of the follicle at ovulation. The theca interna develop LH receptors and are the cells later producing ovarian hormones upon LH stimulation. At this stage the granulosa cells start to express FSH and estrogen receptors and progress into a preantral follicle. The receptors enables the follicle to respond to FSH and LH stimulation and to produce ovarian hormones. The preantral follicles represent the pool of available follicles that can be recruited for further maturation by FSH stimulation. The following stages are gonadotropin dependent and do therefore only happen after the hypothalamus-pituitary connection is fully develop in puberty.

In between the granulosa cells, cavities start to form with follicular fluid containing hormones secreted by the granulosa cells as well as hormones like FSH from the circulation. In the early antral follicle these cavities eventually merges into an antrum creating a confined environment around the oocyte. With the follicular hormone production the growth is accelerated due to positive auto- and paracrine feedback loops by estradiol. This include up-regulation of FSH and estradiol receptors and consequently increased responsiveness of the follicular cells and promotion of further proliferation, growth, and hormone secretion. The antrum grows throughout different stages of antral follicles and create a hormone rich environment required for oocyte maturation. In the presence of both FSH and estrogen, LH receptors are induced at granulosa cells of large follicles. These follicles can therefore also be stimulated by LH causing same effect as FSH in the granulosa cell. Due to this extra source of stimulation and to the escalating self-stimulating growth events caused by estradiol the most advanced follicle will gain further dominance. It hereby become the dominant follicle selected to continue maturation to a preovulatory follicle while less developed follicles undergo atresia. LH is crucial for the final maturation of the dominant follicle as it ensures a supply of androgens from the theca cells required for estradiol production, and it supports stimulation of the granulosa cells to sustain growth and hormone production while FSH levels

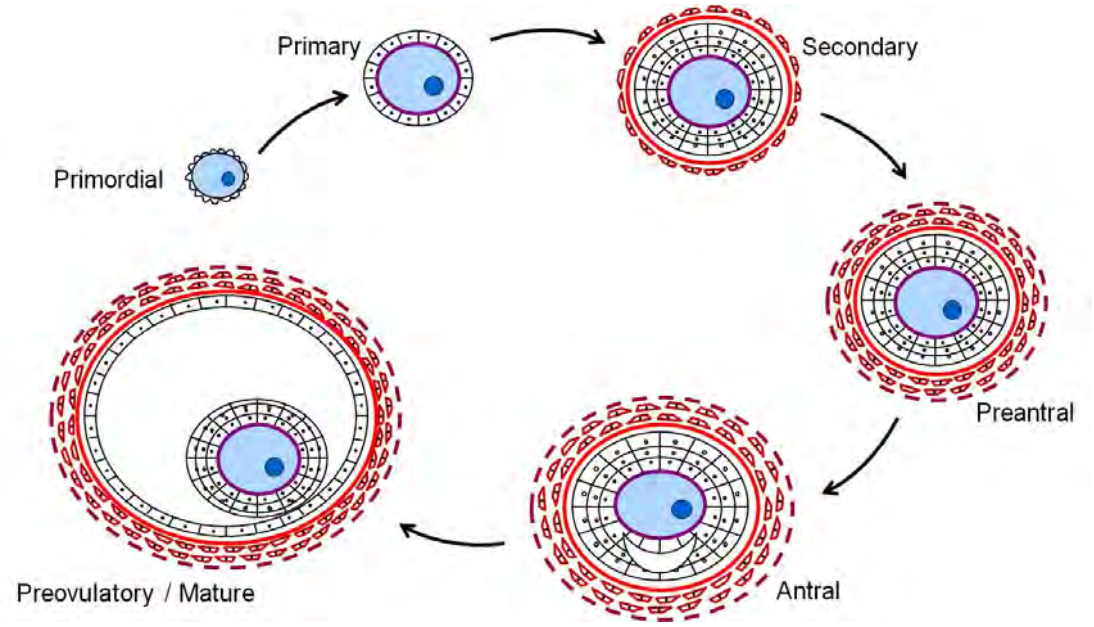

Figure 3.2: Illustration of the follicular development from a primordial follicle to a mature preovulatory follicle. The oocyte is initially surrounded by a single layer of granulosa cells. In the development the layers of granulosa cells increase, theca cells layers are added, and lastly the antrum is formed - a cavity with hormone-rich follicular fluid.

decline. Induction of LH receptors at granulosa cells are therefore essential in the preovulatory follicle. Hence, selection of the dominant follicle to continue maturation of the final stages to ovulation depends on the follicle's estrogen content in the follicular fluid, the induction of LH receptors, and high number of FSH receptors. The granulosa and theca cells change in formation in the final step along with numerous intracellular changes preparing the follicle for rupture. The LH surge prime the oocyte of the preovulatory follicle to initiate completion of maturation and cause luteinization of the granulosa cells. With the release of the oocyte the remnants of the follicle form the corpus luteum.

3.2.1 FSH Threshold

A certain level of FSH is needed to recruit the cohort of preantral follicles for further development. This threshold theory was first proposed in 1978 by Brown (Review [35, 43]). The FSH level needed to sustain growth is lower than that required for initiating growth hence the controlled period with elevated FSH level ensures that only a small cohort starts to grow. There is a different threshold level for each follicle and it can change as the follicle grow and determine the faith of the follicle. Evidence suggest that the dominant follicle is more sensitive to FSH

and has a lower threshold than the others and thus survive a decline in FSH level, whereas the smaller follicles in gonadotropin dependent development will undergo atresia as the FSH level falls below their thresholds.

The duration of elevated FSH level above the threshold is also important. A short elevation by exogenous administration of FSH does not interfere with the selection of one dominant follicle, but a moderate elevation for a longer time cause multiple follicles to continue growing to the mature state [44]. The results supported that more mature follicles become more sensitive to FSH stimulation and thus gain dominance. The duration also affect the number of follicles initially starting to grow. Therefore a FSH window rather than a threshold has been proposed (Review [45]).

FSH levels and individual thresholds play an important role in not only recruitment of preantral follicles, but also selection of the dominant follicle, which follicles continue to grow, and which undergo atresia.

3.3 Menstrual Cycle and Hormone Dynamics

The menstrual cycle lasts on average 28 days and consists of two phases of approximately 14 days: the follicular phase and the luteal phase. Ovulation marks the transition between the phases, and menstruation causes the cycle to start over (Review [33, 34]). Complicated hormone dynamics, as illustrated in Figure 3.3, control the events throughout the cycle.

The follicular phase is initiated by an increase in FSH concentration that allow the group of preantral follicles to grow. As the growing follicles start to produce inhibin B, the FSH level decrease due to the negative feedback. Inhibin B is predominantly secreted in the early to mid follicular phase by the small follicles and thus decrease in the late follicular phase when more and more follicles become atresic. The onset of estradiol production starts later since secondary follicles with theca cells have to be formed. Unlike inhibin B, estradiol production increases with the growing follicles. The dominant follicle produces estradiol in continuing increasing amounts, which causes estradiol levels to rise rapidly. The negative feedback of inhibin B at first and later by estradiol at FSH secretion adds to the advancement of the dominant follicle and atresia of less FSH sensitive follicles. On the contrary, estradiol's effect at LH changes from inhibitory to stimulatory and consequently LH levels begin to rise in the late follicular phase. The maturing preovulatory follicle secrete low levels of progesterone that augment the positive feedback of estradiol at LH production and cause the midcycle increase in FSH as well. This burst of FSH ensures completion of implementation of LH receptors at the granulosa cells and final maturation.

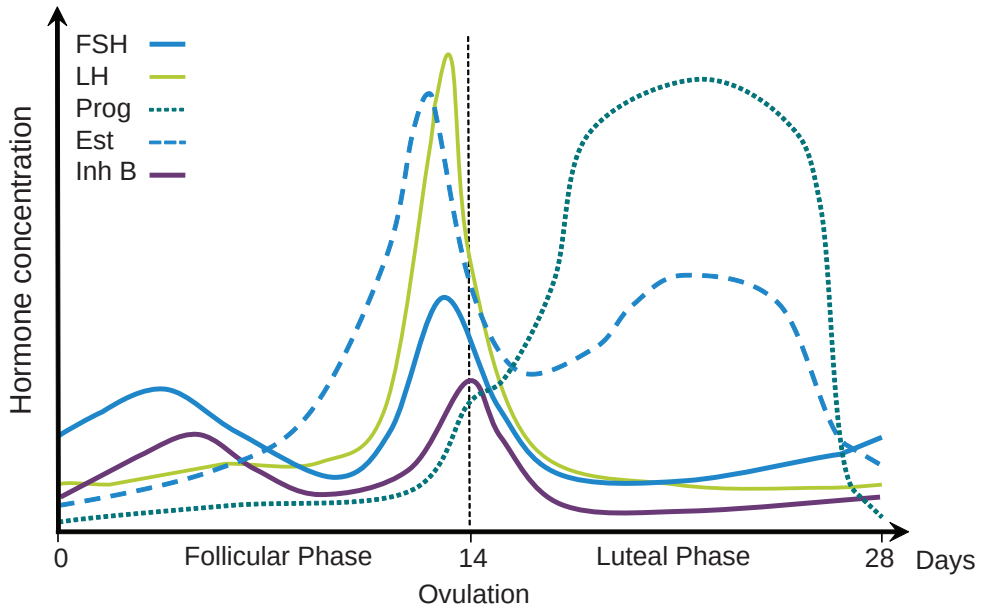

Figure 3.3: Illustration of the reproductive hormone concentration dynamics through the menstrual cycle. The scales are arbitrary and the magnitudes are not comparable between the hormones only the dynamics relative to each other. FSH: follicle stimulating hormone, LH: luteinizing hormone, Prog: progesterone, Est: estradiol, Inh B: inhibin B.

The timing of ovulation is controlled by the maturing follicle and the levels of secreted steroid hormones by the follicle indicate when it is mature and ready for ovulation. The low level of progesterone contributes to the timing of the LH surge that cause ovulation and the beginning of the luteal phase. With the expression of LH receptors at granulosa cells, LH takes over stimulating progesterone production and changing the inhibin production from inhibin B to inhibin A. In addition the LH receptors enable LH to cause luteinization of the granulosa cells. Without FSH stimulation and rupture of the of the dominant follicle the estradiol levels decrease. An inhibin B peak is observed just after ovulation and is most likely caused by the ruptured dominant preovulatory follicle and release of its hormone-rich follicular fluid. After ovulation the remnants of the follicle is stimulated to progress into the corpus luteum which is an endocrine gland producing high levels of estradiol, progesterone, and inhibin A under LH stimulation. Thus inhibin B levels are low after ovulation and hereby the suppressive effect at FSH is diminished. However, FSH concentrations are still low due to suppression from the increasing estradiol and progesterone levels. Progesterone blocks the GnRH surges in the hypothalamus and suppresses the LH release in the pituitary which causes the rapid decline in LH concentration after the surge. If no fertilisation of the ovulated egg occur

the corpus luteum degenerates and the progesterone and estradiol levels decline allowing FSH levels to increase in the late luteal phase. This increase in FSH is important for the initiation of the next cycle.

Chapter 4

Gonadotropin Therapy

Since the discovery of gonadotropins' role in reproduction, several sources and methods for measuring and extracting the hormones have been tested and developed in different directions. Purity, batch-to-batch consistency, tolerability, safety, high potency and activity were desired properties of the preparations for effective infertility treatment. However, the method and source also needed to be eligible for large scale production, without being too complex or expensive. Several products are marketed today but the search continues for better products and innovative treatment strategies.

Cause of infertility varies greatly, it is therefore important to diagnose the patient to determine if the patient at all is eligible for gonadotropin treatment. In addition, it is preferable to find an optimal individual dosing scheme according to patient-specific factors to increase success rate in pregnancy. For this is needed reliable determinants of ovarian reserve and response to treatment. If a good predictor can be identified, there could be therapeutic value in adjusting individual doses according to its level. Thus knowledge of what hormones, factors, and personal demographics that have potential in diagnosing or predicting infertility extend and response is necessary.

4.1 The Road to Recombinant FSH Preparations

For nearly a century gonadotropins have been extracted from various sources including animal pituitaries, pregnant mares' serum, human urine, and human postmortem pituitary glands. When it became possible to identify, isolate, and assess the amount of the hormones, more could be learned about variations in hormone levels within and between individuals and causes hereof. In addition, the gonadotropins' effects could be tested when administered in animals, as well as in humans with intention of developing gonadotropin preparations for infertility treatment.

4.1.1 Human Gonadotropins

Animal preparations could be used in humans and successful treatments were reported, but formation of antibodies was also detected (Review [41, 46]). It was suggested that if treatment was carried out while carefully monitoring individual follicular development and with an improved two phase method, animal products could still be used [47]. With time several groups concluded that to avoid formation of antibodies, gonadotropins had to be of human origin [48–50]. Despite this, animal preparations were used for several decades before they were withdrawn (Review [41, 51], ([51] is mostly a reprint of [52])).

Much was learned from the animal extraction methods that could be adapted to preparations of human gonadotropins. In 1949 Li et al. [53] extracted FSH from sheep pituitaries. When administered to rats it stimulated follicular development and showed no effect of any other hormones than FSH, suggesting purity of the pituitary FSH extract. It took another decade before a similar method was used to extract human pituitary gonadotropin (HPG) from human autopsy pituitaries as starting material for purification and separation of FSH and LH [54]. A partially purified human pituitary FSH preparation with high activity showed promising clinical effect in 7 female patients [55]. At that time it was the preparation with the highest FSH activity [56]. It was possible to purify the preparation even further to get an activity over 2000 times higher than urinary derived standard preparations [57]. In the following years several successful studies in women were conducted with HPG [50, 58, 59], but as postmortem human pituitary glands were only available in limited amounts, HPG could not be produced on a large scale. It was used for ovarian stimulation for 30 years, but after several reported cases of Creutzfeldt-Jakob disease HPG was withdrawn from the market (Review [41, 51, 60]).

Another hormone used in gonadotropin therapy is human chorionic gonadotropin (HCG) extracted from human placenta, pregnant women's urine or produced by recombinant technologies. As early as 1931, only a few years after its discovery, the first HCG product was marketed (Review [51, 61]). It is used as a luteinizing factor and does not contain any FSH activity, hence HCG has no effect on the ovaries unless FSH is present or prior treatment with an FSH preparation has occurred [57]. HCG is still used today in combination therapy to induce ovulation and sustain corpus luteum.

4.1.2 Human Menopausal Gonadotropins

Methods to extract gonadotropins from human urine date back to the 1930s but they were tedious and expensive with varying yield and content. The first urinary preparations had very little activity and were toxic to laboratory animals

(Review [62, 63]). Therefore, before gonadotropin preparations could be administered to humans it was necessary to develop methods for hormone extraction and purification from human sources yielding safe and efficient preparations.

The three major urinary extraction methods for gonadotropins were alcohol-precipitation method [64], kaolin-adsorption method [65], and ultrafiltration [66, 67]. When the kaolin-adsorption method was combined with chromatography great improvements were achieved [68]. And when further treating the urine extracts with tri-calcium phosphate the toxicity could be reduced [69]. Urine from both males and females of different ages were used. With both ultrafiltration and alcohol-precipitation methods it was possible to quantify the hormone amounts in urine to differentiate between normal levels, hyper- and hypogonadotropic syndromes [70]. Menopause is one of the hypogonadal syndromes with increased gonadotropin excretion into the urine. After administration to both intact and hypophysectomised immature rodents it was also observed that extracts from postmenopausal women's urine have higher gonadotropin activity than urine from normal females [71]. In addition it became clear that human menopausal gonadotropin (HMG) contain both FSH and LH activity [72, 73]. Postmenopausal women's pituitary glands and urine were also reported to have higher FSH content compared to other ages and males [74]. Based on these findings it was concluded that extracting HMG from post-menopausal women's urine give higher yield of gonadotropins and is thus the best urinary source for gonadotropin preparations (Review [63]).

Several of the urinary extraction methods were discussed to be used for a standard HMG reference preparation, which was needed for comparison in development of new and hopefully better methods. Not only one, but several standard preparations were established and research groups used different standard preparations as reference [75–79]. A clear comparison between studies was therefore still not possible but it did add some to a more systemic review of preparations. New purification methods were developed to get overall substantially higher activity than the references and obtain a purified HMG safe for human injections [80, 81]. Comparative studies of different HMG preparations suggested that both a certain level of FSH and FSH/LH ratio was needed for an appropriate ovarian response [82].

In the 1960s the clinical use of HMG, often in combination with HCG, was extensive. Administration of HMG in women with amenorrhoea or anovulation induced ovarian response and ovulation with numerous pregnancies following treatment [83–87]. These studies confirmed FSH as being the important therapeutic factor for clinical effectiveness. In the first HMG preparations FSH and LH only accounted for 5 % of the total protein content. In addition, FSH exists in the body in different isoforms and the proportion of isoforms changes over time causing vary-

ing isoform profiles in the urinary extract (Review [36]). Hence these preparations had low bioactivity and high batch-to-batch variability with large amounts of urinary proteins and unidentified substances potentially causing side effects [88]. As a consequence, HMGs were only available for intramuscular (i.m.) administration.

Further development in the fabrication techniques and by applying methods used at pituitary tissue enabled separation and achievement of a partially purified FSH from postmenopausal urine [89]. Starting with existing preparations and applying different methods Donini et al. [90] managed to increase potency and FSH/LH ratio, and by binding and removing LH with chromatography they attained an apparently pure FSH preparation [91]. At last was achieved a highly purified FSH product with higher content of FSH (about 95 %) almost without any polluting proteins [88, 92]. As a result of the lower contaminating content, risk of injecting site reactions were reduced, the bioacivity became higher, thus lower amounts needed to be injected and subcutaneous (s.c.) injection was made possible.

4.1.3 Recombinant FSH

In 1989 advances in DNA technologies enabled production of recombinant FSH (rFSH) from Chinese Hamster Ovarian (CHO) cell lines [93]. This controlled fabrication process eradicate all non-specific proteins normally found in the urine, rendering a pure FSH preparation completely deprived of other gonadotropins as well. Adverse effects and allergenic reactions were thus reduced making it suitable for s.c. administration. In addition, specificity and batch-to-batch consistency increased and the source is sustainable hence can meet any market demands (Review [94, 95], ([95] is mostly reprinted in [96])). When devoid of LH activity rFSH preparations allow for controlled pure FSH monotherapy. This is an advantage as many patients have sufficient LH concentration and do only need FSH for proper ovarian stimulation. Mixed gonadotropin preparations can in these patients even cause adverse effects and reduce success rate in fertilization and pregnancy because elevated LH concentrations can inhibit the stimulatory effect of FSH on the ovaries. High levels of LH can cause off-timed ovulation resulting in poor quality embryos and early pregnancy termination. Only hypogonadotrophic hypogonadism patients need exogenous administration of LH for proper estradiol production (Review [35, 97–100]). Hereby the disadvantages with urinary products were overcome.

Biological characteristics of rFSH are similar to pituitary and urinary FSH preparations but the isoform profile is more consistent and resemblant of the natural circulating FSH with greater number of basic isoforms. Consequently, the potency and specific FSH activity of rFSH are higher [101–104]. In the following years numerous studies were conducted with different rFSH products, doses and

routes of administration. Clinical use of rFSH proved to be safe with promising outcome [105, 106]. The pharmacokinetics of rFSH were comparable with urinary FSH but efficacy and pregnancy rates were higher [107–109]. However, in a meta-analysis of six studies higher clinical pregnancy rates were reported with HMG than with rFSH in a long treatment protocol, but they could not conclude if it was the case for ongoing pregnancy rates and live births [110]. It was generally believed that rFSH would be the future in infertility treatment.

The discovery of the impact of different isoforms and glycosylation on FSH activity and clearance led to development of a long acting rFSH (Corifollitropin alfa, Elonva) that is safe and effective with increased bioactivity and sustained half-life [111–115]. Other research groups have also used different techniques to develop a long acting rFSH [116].

Today existing marketed rFSH products include Gonal-F [117], Puregon [118], and Elonva (Corifollitropin alfa) [115]. Following the marketing of the products other companies have made biosimilars, that have the same active substance as an existing approved biological medicine which is used as reference. Bemfola [119] and Oveleap are biosimilars to Gonal-F, and Fertavid is the same as Puregon. All these drugs are approved by EMA. Gonal-F and Puregon (with the name Follistim) are the only ones approved by FDA as well. A novel rFSH, FE 999049, is under development at Ferring Pharmaceuticals. FE 999049 differs from the other rFSH product as it is expressed in a cell line of human fetal retinal origin (PER.C6®). It has been demonstrated that FE 999049 has different PK and PD properties compared to Gonal-F [120]. To achieve optimised infertility treatment and to increase pregnancy rates it is intended to investigate the possibility of individualised FE 999049 dosing.

4.2 Individualised Treatment

Gonadotropin therapy cannot be used for every type of infertility, thus a diagnose is needed prior to treatment. If the cause is primary ovarian failure, administration of gonadotropins is of no use. Patients with intact ovaries and follicles but who suffer from e.g. oligo-anovulation, inadequate ovarian response, insufficient amounts or imbalanced ratio of gonadotropins may benefit from gonadotropin treatment. That is, the cause need to be functional and not ovarian failure such that the ovaries are responsive with primordial follicles for FSH to exert its stimulatory effect.

Remarkably early in the gonadotropin therapy history it was known that not all infertile patients could become pregnant using gonadotropin stimulation [46, 50, 70]. In addition there was awareness of possible benefits of individualised treatment and dose adjustment according to patients' characteristics [47, 85, 86, 121]. Already in the first study with HPG it also became clear that several injections

were needed for prolonged exposure to achieve proper therapeutic stimulation [55]. A single high dose of 375 IU urinary FSH elevated endogenous FSH levels by 1.9 times compared to normal levels but it was still just a single dominant follicle that reached full maturation. If instead a low dose, 75 IU of recombinant or urinary FSH, was given continuously in the follicular phase of the menstrual cycle, growth of multiple follicles was induced [44, 122]. Thus, suggesting that duration of exposure is just as important as the dose itself for multiple follicular development.

Studies comparing treatment with current rFSH products indicate that different doses may be needed for individuals. Increasing daily dose of Puregon from 150 IU to 250 IU gave only a small increase in oocytes retrieved and the number decreased with age [123]. Even though the higher daily dose shortened the treatment period the total overall amount used was higher. In both dose groups some women had insufficient ovarian response and some women were at risk of hyperstimulation. Another study compared multiple doses of 150 IU with 225 IU Gonal-F in young and older women. They found similar results for oocytes retrieved between dosing and age groups, and concluded that a higher rFSH dose did not compensate for a reduced number of follicles in older women [124]. These results reveal that women within same age group and with similar personal characteristic do not respond in the same way to treatment.

Awareness that one protocol does not fit all, but there is a need for individualisation and knowledge of the influential factors on ovarian stimulation is increasing. It is necessary to identify poor and excessive responders to optimise pregnancy rates and reduce cancellation rates. It has even suggested to be unacceptable to start treatment without knowing the individual patient's potentials and risks. (Review [43, 125, 126]).

The necessary dose and treatment length should vary according to individual response in order to get the FSH concentration within the threshold window for prolonged time to induce proper follicular development and not risking ovarian hyperstimulation. What dose to give, how to identify it, based on which criteria, and if it should be changed during the treatment period are major challenges for individualising infertility treatment.

4.2.1 Predictors of Ovarian Response

Diagnosing the cause of infertility is not always possible. As a minimum it can be checked if the patients have appropriate ovarian reserve with primordial follicles eligible for stimulation. Furthermore, indicators for ovarian sensitivity and magnitude of response would be useful for selecting the individual dose. Fertility decreases with age and comes to a hold at menopause. This is due to changes in hormone productions and decline of the primordial follicle pool since birth. Hormone levels or follicle number could therefore be a better indicator for fertility

than age itself. It is greatly debated which hormone levels and indicators of ovarian reserve are reliable, and which can be used as predicting treatment outcome.

There exists over 20 tests using hormone levels, patient history, and more complicated tests for measuring ovarian function and response to certain stimuli (Review [127]). In a large systemic review from 2006 [128] where the predictive capability of available ovarian reserve and response tests were evaluated, it was concluded that all tests only performed modest to poor. Basal FSH, inhibin B, and antral follicle count (AFC) was though judged to be potentially useful as initial screening test. There were only included two studies using anti-müllerian hormone (AMH) as predictor. The conclusion for AMH was therefore not final and as it performed moderately, further studies were suggested for obtaining evidence of its potential to perform better than the other tests.

Previously it has been shown that elevated FSH baseline is an indicator of reduced response to gonadotropin treatment. A reason for this is probably that the threshold has increased and hereby is needed a higher dose to achieve a sufficient circulating FSH level for ovarian stimulation (Review [43]). Howles et al. [129] proposed after investigating 15 potential predictive factors that dose should be based on not one but four factors: basal FSH concentration, body mass index (BMI), age, and AFC. This algorithm was subsequently used in a pilot study yielding good results for oocytes retrieved and pregnancy rates. They suggested that further adjustments of dose could reduce cancellation due to hyper- or suboptimal response [130]. Other studies support that a combination of AFC and a selection of hormone levels such as FSH, estradiol, and/or inhibin B optimise the predictive information instead of using only one marker [131–133]. A problem with these can be the complexity of assessing all variables and calculating a dose based on the findings. It would set high demands to the clinic in form of ability to perform all the necessary tests, analyse the samples, evaluate results, and execute the calculations by entering the appropriate values into an algorithm.

As more is learned about ovarian function and hormonal influence the superiority of AMH is gaining evidence as an indicator for ovarian reserve (Review [126, 134]) and as a consistent predictor for poor response as it decrease with age and ovarian function and correlates with AFC, which also has high value as a determinant [135–137]. Results from several studies have shown that poor responders have lower AMH level, and some also found that they additionally have lower AFC, higher FSH levels and age than normal responders. Confirmatory to this it is also observed that high responders have higher AMH level, higher AFC and lower FSH levels than normal responders [133, 138–141]. Using AMH level as an ovarian reserve test is believed to have advantages as it is easy to accurately access and for low levels AMH has low variation during the menstrual cycle. In a cohort study with 20 women [142], AMH levels above 1 ng/mL indicated a young well-

functioning ovary and the AMH level varied during the menstrual cycle. Whereas women with low almost steady AMH levels also had lower inhibin B levels and shorter menstrual cycles, and was identified as having an aging ovary with impaired function. It was therefore suggested that if AMH is measured at any day to be below 1 ng/mL it imply reduced ovarian reserve.

Even though several studies point at AMH as the best single predictor of response and treatment outcome the other factors are still in question. Often baseline measurements have been used in assessment of the predictive value of hormone levels, but having markers reflecting the degree of response during treatment can be useful. Inhibin B has the potential of being a predictor for ovarian response because inhibin B levels correlates with follicular development and has been identified as the first PD marker to change after gonadotropin treatment [42, 143]. An increase in inhibin B levels is the earliest hormone increase observed, the increase is steeper and a maximum level is obtained faster than the other hormones. Inhibin B's role as a potential predictor is further supported by that the measured inhibin B baseline and more significantly the rise in inhibin B 24 hours after gonadotropin administration are both higher in good responders [133, 144]. Furthermore, inhibin B levels 24 hours after rFSH administration correlates with AFC and oocytes retrieved [145]. Several studies have investigated how the change in inhibin B during the treatment correlates with treatment endpoints to establish its role as a marker for ovarian response. Number of oocytes retrieved correlates with the increase in inhibin B the first day of treatment in down-regulated subjects [145], with inhibin B levels at days 4-6, 7-8, and 9-10 during treatment [42], as well as with inhibin B levels at day 6 and 8 and the difference between the two days [146]. A threshold of 300 pg/mL inhibin B increase from day 6 to day 8 was also proposed to differ between poor (below the threshold) and normal responders [146]. Others have suggested a connection between inhibin B, AMH and oocyte quality based on findings that the inhibin B rise from day 3 to 4 and AMH correlate with both oocytes retrieved and number of eggs fertilised [132].

Another way to personalise treatment is to calculate dose according to personal demographics that do not give a measure for infertility but instead affect the PK or PD properties of the drug. Giving dose per body size, either measured as body weight, BMI, or body surface area, is a common dosing protocol for many drugs. In gonadotropin therapy it seems like body weight has been chosen as the measure to describe body size influence at the necessary gonadotropin dose. Studies with urinary and recombinant FSH have shown a correlation between serum FSH or the PK parameters and body weight [106, 147, 148]. In recent years dosing Corifollitropin alpha based on body weight has also been proposed [149–152].

When an understanding of what predictors may be important is established,

they can be incorporated in the clinical study protocol to ensure the wanted measurements of the factors are available for further testing its influence on treatment. With sufficient data generated for potential predictors the significance can be tested or validated in the analysis and modelling of data. Using the developed model for simulations different scenarios can be tested for different values of the identified factors. Subsequently, when an influential predictor has been identified with certainty, criteria can be set for initial dose stratification, goals or adjustments during the trial.

From a modelling and simulation perspective influence of all available factors can be tested, but for creating the recommended dosing strategy the complexity need to be considered as dosing should be guided by an easy to use table. It is not durable if the clinicians have to perform numerous complicated tests and measurements, analyse the results and run an advanced computer program in order to find the dose for a patient. It is therefore important to have both knowledge of the biological system and what has clinical relevance when developing models.

Chapter 5

Pharmacometric Modelling

The pharmacometric discipline in drug development utilises mathematical models and simulations based on knowledge of the physiological system, the disease, and pharmacology. The models are used to quantitatively analyse, describe, and clarify results from clinical trials in order to better understand the drug's PK and PD properties. Important knowledge, maybe otherwise inaccessible, can be achieved through analysis of the models and by performing model simulations to test theories and scenarios.

Drug exposure and response often differ between subjects after dose administration in a clinical trial. If a variation is observed between individuals in a study population it is important to investigate the causes of the variation and how it can be quantified and incorporated in the model parameters.

Modelling is an interdisciplinary field, therefore, knowledge from multiple scientific areas is needed to develop a valid and useful model with justified assumptions to obtain a simplified representation of reality. In the previous chapters the background knowledge needed for this PhD thesis has been presented and include the dynamics and function of the reproductive hormone system, the infertility therapy area, and what biomarkers that are relevant to observe to register treatment effect.

In addition, is needed an understanding of methods and software for data analysis, model development, estimation, and evaluation. These modelling techniques are introduced in this chapter with focus on specific methods for model implementation in the software NONMEM [153].

5.1 Population Approach

It is important to study the drug in the intended target population, but this is especially difficult for vulnerable sub-populations like some elderly, paediatric, or intensive care patients. In addition, samples are costly and complicates the logistics of clinical trials since patients have to stay longer at the clinical. Therefore, data from clinical trials do not always include a high number of samples per individual and thus poses challenges in obtaining useful information about the drug from the clinical data. The analysis of such data requires special techniques and software [154–156].

When analysing clinical population data the objective is to describe the typical behaviour of the study population as well as variability between subjects. Previously, standard analysis approaches were naïve pooling of data and the standard two stage approach [157]. Naïve pooling can be used when data contain very few samples per individual. All data is analysed together disregarding which individuals the samples come from. Hereby is only obtained information about the typical parameter values for the population as one unity and nothing about variations in the population. In the two stage approach subjects are analysed one by one to obtain individual parameters and require an extensive number of observations per individual. Average population parameters are then calculated as mean of individual parameters with respective variances to describe the population variability. The advantages with these method are that they are simple, familiar, and straightforward. However, they have been shown to result in bias of the parameter estimates when data is not suitable and because they do not differentiate between types of variability, if at all considered.

Observational data from clinical settings, besides potentially being sparse, can be nonstringent, nonhomogenous, and imbalanced, hence not suited for restricted approaches [156]. New analysis methods were therefore needed. A more efficient way for analysing population data is using nonlinear mixed effects modelling to quantify the PK and PD properties of the drug. With this method data is pooled for estimating population PK parameters but, as opposed to naïve pooling, it is explicitly tracked which individuals observations belong to, in order to obtain the variability and distribution of individual parameters [157]. The population approach with nonlinear mixed effects models facilitate identification of variability as well as differentiation between interindividual variability (IIV) and unexplained variability which includes measurement errors, intraindividual variability, and model misspecification. Furthermore, it gives the possibility of quantitatively analyse and identify factors causing the variability, and opens for opportunities to include more physiological correct descriptions for mechanism- or physiological-based modelling. The population PKPD approach is an analysis technique for solving the issue: "How to learn what we need to know to administer drugs optimally in clinical settings" (quote [156]).

An important progress in the population modelling field was the development of NONMEM by Beal and Sheiner [158] - a software for implementing nonlinear mixed effects models and estimating model parameters. Notably with their three articles in the early 1980's on evaluation of methods for estimating population pharmacokinetic parameters [157, 159, 160], Sheiner and Beal sat ground for the population PK approach and showed the great usefulness of NONMEM, even for sparse clinical data, and its superiority to the standard methods. The development of NONMEM started in 1972 and up till his death in 2004, Lewis B. Sheiner played

a pivotal role in this development (for an excellent review see [161]). During the years others have made valuable contribution to the further development of NONMEM [153]. Other software for analysing population data exist (reviews [162, 163]) but NONMEM is the most widely used. Within the first decade of the population modelling approach's existence, more than 40 studies reported results obtained from population modelling [156]. Hereafter published articles concerning population PK and NONMEM per year have increased exponentially [164].

5.2 Nonlinear Mixed Effects Modelling

A nonlinear mixed effects model consists of fixed effects, random effects, and independent variables. In a population PKPD framework the model is made up of three sub-models; the structural model, the statistical model, and the covariate model. The fixed effects are the PK, PD, and covariate effect parameters [156, 165]. The PK parameters describe the processes controlling the time-course of drug concentration in the blood or tissue of interest, and the PD parameters are related to the effect of the drug. The average parameter values in a population characterise a typical individual in the population and are therefore also called typical population parameters. The random effects are divided into interindividual and intraindividual variability, which is the variability between subjects and residual errors, respectively. Covariates, time, dose, and other variables in the study design are independent variables in the model.

Structural Model

A structural PK model consists of a number of compartments involved in the drug's path through the body. The change in drug amount in each compartment is described by a differential equation with structural model parameters, which are the fixed effects in the model and denoted by θ's.

A one-compartment model after an i.v dose is the simplest PK model. It is illustrated in Figure 5.1.a and the associated differential equation is

$$\frac{d\mathrm{A}_1(t)}{dt} = -k\mathrm{A}_1(t) \ , \quad \mathrm{A}_1(0) = \text{dose}. \tag{5.1}$$

The elimination rate, k, is clearance (CL) divided by the volume of distribution (V), and the drug concentration at time t is the amount in the central compartment ($\mathrm{A}_1(t)$) divided by V. The initial condition for the differential equation is dose.

For a two-compartment i.v. model an extra equation is added for the peripheral compartment (A_2) and the drug amount flow from and to the central compartment follows the distribution rate constants k_{12} and k_{21}, respectively. In Figure 5.1.b

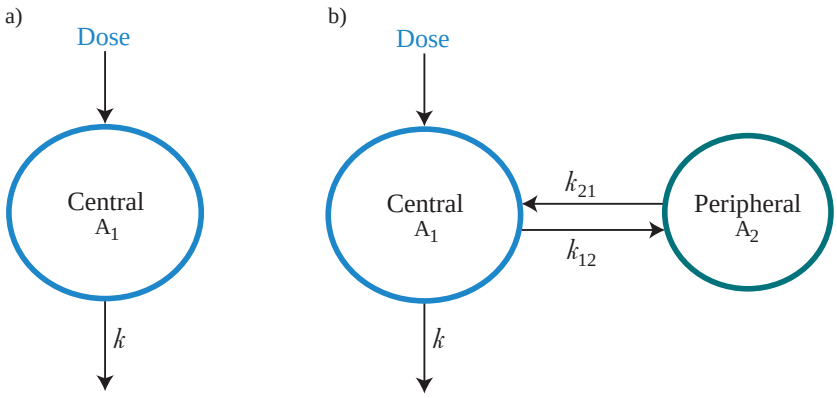

Figure 5.1: Drug distribution after an intravenous dose following a) a one-compartment or b) a two-compartment model.

is illustrated a two-compartment i.v. model with elimination from the central compartment. The dynamical system for the model then becomes

$$\frac{d\mathrm{A}_1(t)}{dt} = -k\mathrm{A}_1(t) - k_{12}\mathrm{A}_1(t) + k_{21}\mathrm{A}_2(t)\ , \quad \mathrm{A}_1(0) = \text{dose}. \tag{5.2}$$

$$\frac{d\mathrm{A}_2(t)}{dt} = k_{12}\mathrm{A}_1(t) - k_{21}\mathrm{A}_2(t)\ , \quad \mathrm{A}_2(0) = 0. \tag{5.3}$$

The terms one- and two-compartment PK models refer to the distribution of the drug, but after other administration routes than i.v. the complete model contain more compartments. When the dose is given orally, s.c., or i.m. a compartment representing the dosing site is included, from where the drug is absorbed to the central compartment with the rate constant k_a. A transit model with extra compartments can be added to describe a delay in the absorption process. An example of this type of model is represented by the compartment diagram in Figure 5.2 with the respective differential equations:

$$\frac{d\mathrm{A}_1(t)}{dt} = -k_{tr}\mathrm{A}_1(t) \tag{5.4}$$

$$\frac{d\mathrm{TR}_1(t)}{dt} = k_{tr}(\mathrm{A}_1(t) - \mathrm{TR}_1(t)) \tag{5.5}$$

$$\vdots$$

$$\frac{d\mathrm{TR}_n(t)}{dt} = k_{tr}\mathrm{TR}_{n-1}(t) - k_a\mathrm{TR}_n(t) \tag{5.6}$$

$$\frac{d\mathrm{A}_2(t)}{dt} = k_a\mathrm{TR}_n(t) - k\mathrm{A}_2(t). \tag{5.7}$$

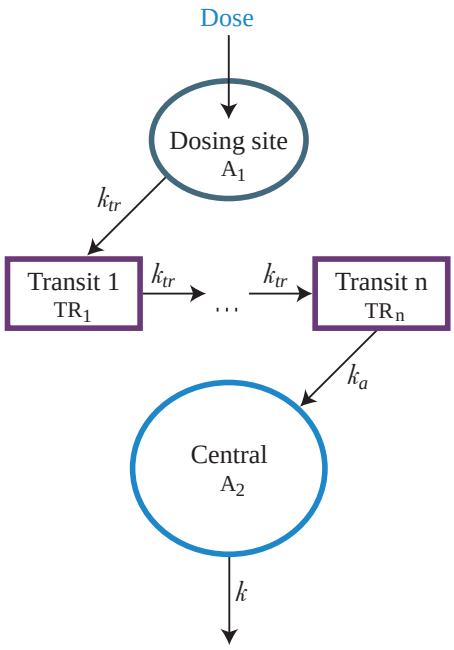

Figure 5.2: A one-compartment model after e.g. a subcutaneous dose. The absorption process from the dosing site is delayed by n transit compartments.

The initial condition at the dosing site is dose, and the others are zero. The rate constant for the flow into the transit compartments is (k_{tr}). In an absorption model the complete dose might not be absorbed, and without i.v. data the bioavailability (F) is not known, hence the PK parameters that can be estimated are the apparent volume of distribution (V/F) and apparent clearance (CL/F). The tradition in the field is to denote this a one-compartment model despite the additional absorption and transit compartments.

The compartments do not necessarily represent an anatomically defined part of the body but rather a collection or a symbolic compartment. With moving from an empirical to a semi-mechanistic and mechanistic approach, parameters and compartments will to a larger degree be representative for physiological processes and anatomical parts. In a physiological-based model all compartments correspond to an organ or tissue in the system of interest and the parameters will have a physiological interpretation.

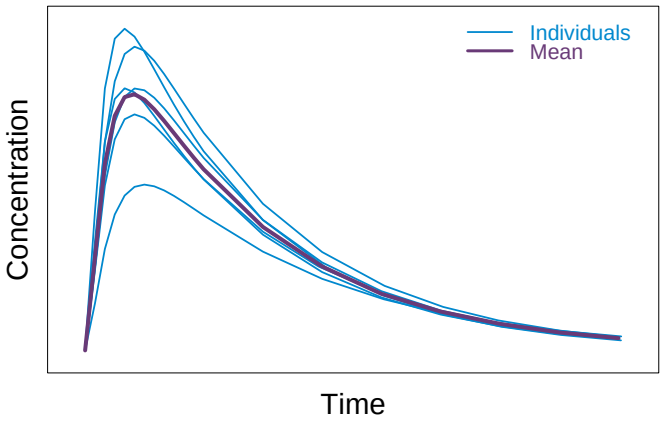

Figure 5.3: Illustration of variation between subjects in a population. The PK profile of individual subjects (blue lines) and the population mean profile (purple line).

Statistical Model

Individual PK profiles are likely to vary from the population mean for a typical individual in the observed population (Figure 5.3). In order to describe this variability between subjects in the population, random effects are added to the typical model parameters where needed. One way to add the random effect for IIV at a typical population parameter (θ) to obtain the i^{th} subject's individual parameter (θ_i) is with an exponential model

$$\theta_i = \theta \exp(\eta_i). \tag{5.8}$$

The individual random effect (η_i) comes from an approximately normal distribution with mean zero and variance ω^2 for describing the IIV of the parameter. The covariance matrix for all IIVs is denoted Ω. The variability between two parameters can be correlated and is incorporated in the model by adding a covariance for the interindividual random effects in Ω. The advantage of this model over e.g. an additive is that the individual parameter cannot become negative.

In addition, individual model prediction at time t_{ij} may differ from the corresponding j^{th} observation y_{ij} by a residual error ε_{ij} (Figure 5.4). It is the intraindividual variability and covers the unexplained model misspecification, such as unknown influencing factors that are not possible to describe in the model. The residual errors are assumed normally distributed with mean zero, variance σ^2, and covariance matrix Σ. Together, the random effects make up the statistical model, which is also called the error model or stochastic model.

A third type of variability may be considered, the interoccasion variability, which can be important to model if subjects are studied at different occasions over time such that the individual parameters can change during the study period [166].

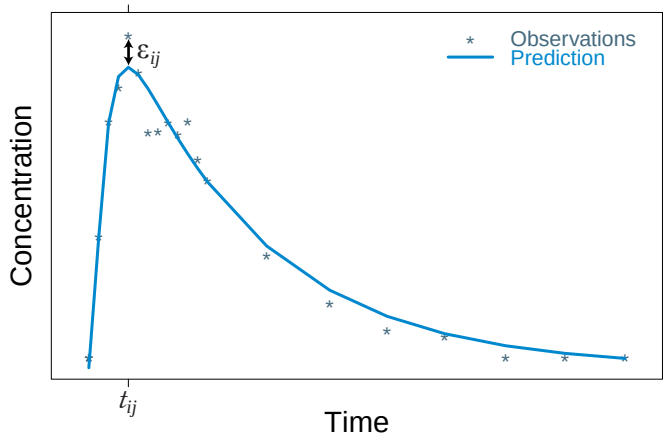

Figure 5.4: The difference between observations (points) and model prediction (line) for individual i at time t_{ij} is the residual error ε_{ij} - a random effect in the model.

Covariate Model

The last part is the covariate model where potential factors influencing the variation in parameters and drug effect are identified. Covariates can for example be personal demographics, disease parameters, or other factors in the target system. Covariate analysis can furthermore be used to identify sub-groups of patients who do not get optimal effect due to distinctive characteristics and therefore would need different doses. Thus, the covariate model will further increase the predictive capability of the model.

In the model potential influential factors can be tested for significance as a covariate to explain some of the IIV in a parameter. Continous covariates can for example be added in a linear, exponential, or power relation, and often the effect is normalised or centred to a standard value of the covariate like population mean or median. A typical covariate at CL and V is body weight, since body composition can affect drug metabolism and distribution. It might be underlying mechanisms responsible for the impact of body weight, but such detailed measurement are mostly not available thus body weight is expressive for the differences. The effect can be included in the model with allometric scaling at the parameters

$$\theta_i = \theta \exp(\eta_i) \left(\frac{\mathrm{WT}_i}{\mathrm{WT}_{st}} \right)^{AL_\theta}, \tag{5.9}$$

where WT_i is the i^{th} subject's body weight, WT_{st} is the normalisation value, and AL_θ is the allometric values: 0.75 for CL and 1 for V. The same expression can be used for other covariates and other parameters, but then AL_θ is estimated as the power exponent for the effect. Other functions like linear and exponential can also

be tested for describing a covariate effect. For categorical covariates (e.g. gender) different θ's are used for the categories and potentially also different η's.

The model prediction for individual i can be presented as a function of time, the other independent variables (x_{ij}), and the vector of individual i's parameters (Θ_i). The individual observations are described by adding the residual errors to the model prediction

$$y_{ij} = f(t_{ij}, x_{ij}, \Theta_i) + \varepsilon_{ij}. \tag{5.10}$$

In general an individual parameter is given as a function of the typical population parameter, individual random effect, and individual associated values of covariates (c_i)

$$\theta_i = h(\theta, \eta_i, c_i), \tag{5.11}$$

where the random and covariate effects potentially are absent.

5.3 Pharmacokinetic-Pharmacodynamic Modelling

PKPD models are not as straight forward and simple as PK models. There do exist a number of predefined effect models but the variation by combinations and alterations of the models gives many options. Three major types of PKPD models are direct link, indirect link, and indirect response models. A few examples will be given here, but for extensive reviews see [167, 168].

Concentration-effect or equivalently named exposure-response models are PKPD models describing the effect caused by the drug concentration and the combined time-course. In direct link models the drug concentration is directly included in an expression for the effect. The site of action where the effect occur is not necessarily the blood (or another body fluid) where the drug concentration is typically measured. Therefore, steady state conditions and equilibrium between the concentration at the effect site and the blood is often assumed in order to directly model the effect of the drug concentration.

It can simply be an all-or-none model where the effect occur above a certain concentration threshold and not below. Or the effect can for example be described by a linear model, a power model, an $E_{\max}$ model, or different sigmoid $E_{\max}$ models with Hill coefficients. The equations can include a baseline effect if an effect is present before any drug is administered. A linear or power model might not seem feasible as the effect has no limit which is not physiological plausible. Anyhow it can in a certain concentration area be the best description of the effect and can be seen as an approximation to an $E_{\max}$ model. Such approximations may be suitable for concentrations (C) much lower than EC_{50}, the concentration where half of the

maximum effect ($E_{\max}$) is obtained. Consequently the sigmoid $E_{\max}$ model with Hill coefficient λ is approximately reduced by one parameter to a power function:

$$\frac{E_{\max}C^{\lambda}}{EC_{50}^{\lambda}+C^{\lambda}} \approx \frac{E_{\max}}{EC_{50}^{\lambda}}C^{\lambda} = kC^{\lambda}, \tag{5.12}$$

with $k = E_{\max}/EC_{50}^{\lambda}$.

If substituting drug concentration with dose in the direct link model, the model becomes a dose-response model, which is a simple direct PD model describing the change in effect with dose and bypassing any variability in the pharmacokinetics.

Indirect link models introduce a time delay for the effect to occur. The delayed response can be incorporated in the model by linking the PK model to the PD effect by an effect compartment. It is a hypothetical effect site that does not affect the drug amount balance in the PK model but represents drug distribution from the central compartment. The PK model is therefore unchanged and the following equation is added for the effect compartment

$$\frac{dC_e}{dt} = k_{1e}C_c - k_{e0}C_e, \tag{5.13}$$

where C_c and C_e is the concentration in the central and effect compartment, respectively. It might not be possible to differentiate between the first order distribution rate constants k_{1e} and k_{e0}, hence they are often set to be equal. The effect compartment concentration is then used in the PD model.

The pharmacodynamic response can be time-dependent when the effect is not directly related to drug concentration. In such situations an indirect response model is needed where the drug concentration indirectly affect the response variable. It consists of a turnover model as the basic model for describing the response (R) over time as a separate differential equation with a zero order production rate constant (k_{in}) and a first order rate constant for elimination (k_{out})

$$\frac{dR}{dt} = k_{\text{in}} - k_{\text{out}}R. \tag{5.14}$$

In the absence of drug $R(0) = k_{\text{in}}/k_{\text{out}}$ initially. An inhibitory or stimulatory function of drug concentration is then added to either of the rate constants [169] to get the change in response over time. Stimulating the input or inhibiting the output cause an increase in the response and inhibiting the input of stimulating the output cause a decrease in the response.

Dose-concentration-response models characterise the full PKPD picture of how the effect changes in relations to drug concentration and in turn to dose and over time.

Irrespective of the type of PKPD model, a sequential or a simultaneous modelling approach can be chosen. In a sequential model the PK model is developed

first and then either the estimated PK parameters or predicted concentrations are used in the PD model. In simultaneous modelling all PKPD model parameters are estimated at once. As this can include many parameters, priori knowledge of the values might have to be used depending on the size of and information in the data. As opposed to sequential modelling, simultaneous modelling gives the opportunity of (semi-)mechanistic modelling with feedback to the PK model.

Prediction is of great importance in clinical drug development and models for predictive purposes should be mechanistic rather than empirical [167, 170]. In addition, population modelling with random effects should be chosen over modelling of only population averages, as it also favours incorporation of physiology. With a mechanistic modelling approach a more accurate model can potentially be achieved for increased precision in predictions and further understanding of the system.

5.4 Estimation Processes

Fitting the model to the data is done by estimation processes searching for parameter estimates that maximises the probability of data (y) occurring under the model: $\mathrm{P}(y|\Theta, \Omega, \Sigma)$. Maximum likelihood estimation methods are commonly used for obtaining model parameter estimates for the best model fit to the data. Given the data and model the likelihood function provides a measure for how likely a specific set of model parameters are. For a nonlinear mixed effects model the individual likelihood of the model parameters for the individual data is defined as the marginal likelihood

$$L_i(\Theta, \Omega, \Sigma|y_i) = \int_{-\infty}^{\infty} p(y_i|\Theta, \eta, \Sigma)\ p(\eta|\Omega)\ d\eta, \tag{5.15}$$

consisting of the density functions of the individual data (y_i) and the individual random effects (η). The log-likelihood function can be used in the estimation process to obtain a more numerically stable function. The log-likelihood for the model parameters for the whole dataset is given as a sum of all individual log-likelihoods

$$l(\Theta, \Omega, \Sigma|y) = \sum_i \log\ L_i(\Theta, \Omega, \Sigma|y_i). \tag{5.16}$$

This integral can rarely be solved analytical so in the software NONMEM the log-likelihood function is numerically approximated by different types of linearisation and iterative procedures [153]. The NONMEM objective function value (OFV) is approximately proportional to -2log-likelihood of data given the model and parameters, and is thus sought to be minimised. Some of the specific estimation methods for NONMEM are first order (FO), first order conditional estimation

(FOCE), and Laplacian method. The FO estimation method was the first available method, in which a first order Taylor series expansion of the log-likelihood around the expected values of the parameters, i.e. without the random effects, is used. The fixed effect population parameters are then estimated by extended least squares. The random IIV effects can be obtained posthoc as empirical Bayes estimates (EBE) of the η's. In the FOCE method the linearisation is made around the conditional estimates of the η's, i.e. η estimates obtained conditionally on the population parameters, and therefore simultaneously obtain population parameter estimates and EBE's. FOCE with interaction takes into account the interaction between IIV and residual error. The Laplacian method is similar to FOCE but uses second order Taylor series linearisation instead of first order and hereby increases accuracy but also computational complexity.

5.4.1 Data Requirements

The dose is not explicitly contained in the differential equations of the model, but given as initial conditions. Therefore, the time of dose administration, amount, and number of doses for each subject has to be included in the data, as well as specifying dose as the input to a compartment in the model depending on the route of administration. Data is allowed to be sparse, imbalanced, and incomplete, because when using the population approach information from all individuals are used, and full individual profiles can be obtained even though there are time points with missing measurements for some individuals. Missing covariate values will have to be dealt with in order to test the effect. Ways of doing this can be to use the mean or median of the population values.

The laboratory equipment and assay for measuring the concentration in the blood samples has a lower limit of quantification (LLOQ) for the lowest measurable concentration that is validated. When this limit is reached the observation is reported as below quantification limit (BQL) in the dataset meaning that the concentration is somewhere between zero and LLOQ. Thus BQL observations do not have a concentration value and therefore need to be changed or removed before the data is used for estimation of model parameters. Depending on the proportion the BQL measurements constitute of data, they might have to be accounted for in the model development to avoid biased parameter estimates. There are seven different ways to handle BQL data points as listed by Stuart Beal in 2001 [171]. There are four simple methods where all BQL observations are either discarded (M1), replaced with LLOQ/2 (M5), or replaced with zero (M7). In the M6 method the BQL observation is set to LLOQ/2 if it follows an observation with a value above LLOQ and it is discarded if it comes after another BQL observation, thus in consecutive BQL observations the first is set to LLOQ/2 and the rest discarded. The M2-M4 methods are likelihood based approaches where the BQL observations

are discarded (M2) or treated as censored (M3,M4). In most cases the preferred method for handling BQL observations is M3 as it gives the least bias, in particular with a high BQL proportion in the data [171–173]. With the M3 method BQL data are censored observations and treated as categorical data. These are included in the likelihood function for the estimation as the likelihood that the observation is truly below LLOQ. Therefore, from the estimation process for these observations a probability is returned and not a value for a predicted concentration [171].

5.5 Model Development and Evaluation

As a guideline for model development the structural model, then the statistical model, and lastly the covariate model is developed. The sub-models are not separate models, therefore significance of previously included of excluded parameters can change. It can take several loops of refinement, challenging, and evaluating previous steps before a satisfactory model is achieved. A proposed modelling structure is developing the structural model with relevant residual errors (first-stage model). The second-stage model is addition of IIV and covariates for obtaining individual parameters [170]. In reality, the residual errors are included in the structural model development as a minimum, but often IIV is also added to some parameters from the beginning when there are clear variations in the population. The interaction between the sub-models have been studied with different datasets, and the sub-models were found to be highly intertwined and the choice of one sub-model affected the other significantly [174]. A revised model building strategy was suggested after this observation. From the structural models evaluated, a simple supportable but not necessarily the best model should be chosen for covariate analysis. If a more complicated structural model was observed to have some advantages, the found covariate model should then be tested with this model. As the structural model has been changed left out covariates should be reconsidered and the significance of included covariates should be retested.

Besides deciding on a modelling strategy, evaluation criteria are needed in order to choose the best model. For a quantitative assessment for choosing between two models and evaluate the model fit both statistical and graphical tools are used. There are shortcomings to the different evaluation methods and it is therefore important to use several for a proper diagnosis and challenge the model by using simulations [175].

5.5.1 Statistical Methods

The difference in OFV between two nested models is approximately χ^2-distributed, with degrees of freedom equal to the number of differing parameters. To determine

which of two nested models are statistically significant best, a χ^2-test can therefore be performed on their OFVs and used for selection of inclusion (or deletion) of a model parameter. For 1 degree of freedom and 5% significance level the decrease in OFV from the reduced model has to be greater than 3.84 for choosing the larger model.

The model selection should not purely be based on the OFV value. A logical evaluation of parameters should be performed to get an overview of if the parameters are reasonable and if they make biological sense when a more mechanistic approach has been used. A measure for a parameter's reliability is the relative standard error (RSE = SE(θ)/θ).

It should also be checked if the assumption of the individual random effects having a mean of zero holds. NONMEM returns a probability for if the true mean of the η distribution for the IIV on a population parameter is zero. This p-value has to be greater than 5%. If the individual information is scarce the phenomenon of η-shrinkage can occur, where the individual parameters shrink towards the typical parameter values [176]. It is defined as

$$\eta_{\text{shrinkage}} = 1 - \frac{SD(\eta_{\text{EBE}})}{\omega}, \tag{5.17}$$

where η_{EBE} are the EBE estimates of the η's and ω is the standard deviation of the η's. In case of high η-shrinkage (above 20 or 30 %), the interpretation of the EBE-based diagnostic plots (presented below) should be made carefully, as false relationships might have been induced. For even higher values, the individual random effect model should be reconsidered as it can also mean that the model is wrong.

5.5.2 Graphical Methods

Several diagnostic and goodness-of-fit plots are useful for evaluating how well the model describe data. Plotting mean of predictions and mean of observations can give an overview of the trend in model fit over time, e.g if the baseline value is over- or under-predicted, or if the model do not catch the change over time. One fault of this plot is it does not show if individuals have different curves that all are poorly predicted but by chance the means match. Observed and predicted PK or PD profiles can be plotted for each individual instead to further inspect model fit.

The visual predictive check (VPC) is a diagnostic plot using simulations to check the model's predictability. First, 1000 datasets for model predictions are simulated using the distribution of the estimated parameters, interindividual variability and model residual errors. Then, observed data is compared to the simulated model predictions by plotting the 2.5th, 50th, and 97.5th percentiles of observations and the 95% confidence intervals (CI) for the corresponding model

prediction percentiles. When observations include BQL data points a two panel VPC for illustrating both continuous and categorical (BQL) data has to be used [173]. The top panel displays the observations above LLOQ like a normal VPC. Since a value is not estimated for BQL data the evaluation of these is instead shown in a second panel as the median observed proportion of BQL observations with the 95% CI for the median of simulations.

Goodness-of-fit plots are the dependent variable versus predictions and different residual plots. In the first type the observed values are plotted against individual predictions or population predictions for a typical individual to get information of the variability in the data. Different types of residuals can be plotted against prediction or an independent variable. Individual weighted residuals are defined as

$$\text{IWRES} = \frac{y_{ij} - \hat{y}_{ij}}{\sigma}, \tag{5.18}$$

where $\hat{y}_{ij}$ is the individual prediction of the individual observation y_{ij} and σ is the error magnitude (standard deviation) of the residual error model. These residuals may not reveal model misspecification and are only useful when individual data is informative [175, 176]. Standard residuals are the difference between observations and population predictions and lack information at the individual level and can inaccurately show residual trends. The weighted residuals (WRES) are normalised to residual variability and thus more explanatory of the model but the calculation is based on the FO estimation method. With the development of improved estimation methods the FO method is rarely used, hence conditional weighted residuals (CWRES) based on the FOCE method are better for diagnostic purposes [177].

Correlations between parameter's random effects can be identified by plotting EBE's against EBE's, and covariate relationships can be investigated by plotting both parameters and random effects against potential covariates. In these EBE diagnostic plots it is important to remember to consider the size of η-shrinkage.

Chapter 6

Modelling of FSH Compounds

The aim of PK and PKPD modelling of FSH compounds in drug development varies from comparing different FSH products, acquire knowledge of the drug's properties, quantify covariate influence, and to dose finding purposes. Treatment strategy and optimal dose can vary greatly between infertile patients due to the great difference in cause and extent of infertility. It is therefore valuable to describe the time course and variations in drug concentration and effects properly, and ultimately be able to predict the outcome of a given dose in a specific woman and thereby provide individual dosing schemes. In this chapter is reviewed which PK and PKPD models of FSH compounds exist, and at the end the foundation for the research questions in this PhD thesis is given.

6.1 Existing Mathematical Models

The pharmacokinetic properties of gonadotropin preparations have been studied extensively, but existing PK and PKPD models for FSH compounds are limited and even fewer population models with nonlinear mixed effects modelling exist.

6.1.1 Pharmacokinetic Models

Several studies by le Cotonnec et al. use modelling to describe the pharmacokinetic behaviour of urinary and recombinant FSH in pituitary down-regulated female volunteers. Two studies investigated and compared the pharmacokinetics of urinary human FSH in a standard form (Metrodin) and in a highly purified form (Metrodin HP) [92, 178]. A series of three connected studies [143, 179, 180] investigated the PK and PD properties of Gonal-F. The first study [179] compared Gonal-F with Metrodin. The second study [180] investigated the pharmacokinetics of Gonal-F after different routes of administration and after multiple doses. The third study [143] used the multiple dose data from the second study to develop a PKPD model (described below).

It was found that recombinant and urinary FSH have similar PK properties. The studies also included non-compartmental analysis where the endogenous FSH was handled by baseline correcting data. The two pharmacokinetic analysis approaches gave similar results.

The four PK studies and type of models are listed in Table 6.1. The models were exponential models where an exponential equation describes the drug concentration in a one-compartment model and a two-compartment model consists of a biexponential equation. If an endogenous FSH contribution was modelled it was simply included as a linear function of time, and an initial baseline value was added to the equation for an intercept. The resulting PK models after s.c. or i.m. administration of both urinary and recombinant FSH were one-compartment models with first order absorption, whereas after i.v. doses a two-compartment model was found to describe data best. These studies describe data well and identify a large variation in the PK parameters, but lack consideration of covariates and alternative models. When using a population approach with nonlinar mixed effects modelling instead of subject-by-subject analysis, parameters with interindividual variation can be specified and it is possible to further investigate causes of the observed variation by covariate analysis.

Four published articles with population PK models developed using the population approach with nonlinear mixed effects modelling and NONMEM software to analyse data after dosing with urinary or recombinant FSH were retrieved (Table 6.2). Karlsson et al. developed population PK models [148] using data from three different studies with infertile patients or pituitary down-regulated healthy female volunteers receiving urinary human FSH (u-hFSH) or rFSH. It was not reported which specific FSH products were used, only the type of FSH. Data from i.m. and s.c. dosing was combined with i.v. data when available for the modelling. After i.m. administration of u-hFSH and s.c. administration of rFSH (study 1) the resulting model was a one-compartment disposition model, whereas after i.v. doses of rFSH (study 2) and u-hFSH (study 3) a two-compartment model was used. These results are in accordance with the previous results with exponential models. It was though found that when the i.m. or s.c. data was combined with the i.v. data for the same subjects, and hereby increasing the number of samples for each individual, a two-compartment model could adequately describe the data. The first study where one-compartment models were identified had few samples per individual. It was concluded that the exogenous FSH pharmacokinetics were best described by a two-compartment model if the data was "rich", meaning that there were an extensive number of samples. The model structure does not depend on the route of administration as it is related to the properties of the drug but it is the data that dictates how many compartments that can be characterised. The consistency in number of compartments in the model with route of administration was therefore due to the richness of data.

Covariate analysis was only performed in the first study dataset with patients receiving u-hFSH or rFSH. An influence of body weight at CL was significant in the model with the u-hFSH data. BMI was a covariate at k_a and creatinine clearance

(CL_{CR}) at FSH baseline in the rFSH data. In both models the FSH baseline was found to be best described by an exponential decline over time. The second dataset with healthy female volunteers given rFSH was divided into four subsets (see Table 6.2) and four models were developed with different extent of linear decline in FSH baseline over time. In the last dataset with single dose u-hFSH the baseline was almost constant over time.

Three studies investigating the dose-response relationship and pharmacodynamic properties of Corifollitropin alfa (a long acting rFSH) after a single s.c. dose in infertile patients [149, 181, 182] used population PK models and NONMEM to estimate PK parameters. Development of a pharmacokinetic model was not the primary objective in these studies, thus no details about the development was provided. A one-compartment model with first order absorption was used, and in two of the studies [149, 181] body weight was included as a covariate at CL and V. Another article [183] with Corifollitropin alfa used a population PK approach to analyse data, but it was purely result oriented purpose and only the PK parameters obtained from the model were presented. Non-modelling studies with rFSH products have found a negative correlation between serum rFSH levels and body weight [106, 147] further supporting the role of body weight as a factor in determining the pharmacokinetics of FSH compounds.

6.1.2 Pharmacokinetic-Pharmacodynamic Models

A few PKPD models for urinary and recombinant FSH are listed in Table 6.3. Karlsson et al. [184] used a population PD model to predict the follicular growth in patients after dosing with u-hFSH and rFSH. It was tested whether FSH concentration could be included in the model as a separate term with either the total endogenous and exogenous FSH concentration, baseline corrected levels, or as normalised to pretreatment levels. Numerous models and covariate relationships were evaluated. The change in total follicular volume (TFV) was found to be independent of FSH concentration, and the best model was an $E_{\max}$ model for TFV plus a constant term. It was possible to explain some of the interindividual variability in the $E_{\max}$ model parameters with pretreatment FSH level and baseline estradiol.

Inhibin B and estradiol levels have been described after multiple s.c. doses of Gonal-F in pituitary down-regulated healthy female volunteers with a sequential PKPD model [143]. The PK model used had been developed in a previous paper [180] (described above) from where the estimated PK parameters were fixed in the PKPD models.The PK model was linked to the PD models with an effect compartment and the PD responses were calculated from the FSH concentrations in the effect compartment. Both PD models describing inhibin B and estradiol concentrations were power functions. They did not use a population modelling approach but fitted the model to individual data in order to obtain the PD pa-

rameters. A large interindividual variation in the estimated inhibin B response and parameters were observed but since no covariate analysis were performed, no factors causing the variation were identified. A high correlation between TFV with both maximal inhibin B and with estradiol levels was found, but there was no correlation between FSH concentrations and any of the maximal effects for the PD markers. Thus, the high variation between subjects in PD parameters was not due to pharmacokinetic variations but different pharmacodynamic sensitivity. It is therefore not enough to adjust dose after variations in FSH concentrations but the response should also be taken into account. These findings of relation between PD responses and large PD variation were confirmed with a new dataset [185], where almost the same PKPD models were used. The only adjustment was due to a measurable FSH baseline concentration and therefore a constant term for the effect at baseline was added to the power function in the PKPD models. In addition was found that large fluctuations in FSH baseline production over time influenced the pharmacokinetics indicated by high variation in the individual parameter estimates and in predicted FSH concentration at late time points, where the FSH concentration is mostly due to endogenous FSH.

The fourth and last PKPD article found is a more extensive but still empirical model for Corifollitropin alfa by de Greef et al. [151]. A population PK model and four PD submodels were generated sequentially and then combined to predict ovarian response and used for optimal dose selection. NONMEM was only used to develop the PKPD model for Corifollitropin alfa (PK) and inhibin B (PD) concentration-time profiles. For the last three PD models for follicular volume, cancellation rate, and number of oocytes were used SAS. The PK model was based on 1263 Corifollitropin alfa concentrations from three clinical trials with single s.c dosing (in fact the three mentioned above with PK models [149, 181, 182]). Both one- and two-compartment models were tested, but since the data had sparse sampling a two-compartment model could not be fitted to data. Of the potential covariates: body weight, BMI, height, and age, was body weight at CL and V the only significant relationships. The difference from the above two models is that the inhibin B concentration is linked to the predicted Corifollitropin alfa concentration by an indirect response model. In which the production of inhibin B is stimulated by individual Corifollitropin alfa concentrations following a sigmoidal E_{max} function and the elimination over time is also modelled explicitly in the differential equation for inhibin B. The model development was guided purely by data and to correct for an undershoot in inhibin B below the baseline values, a hypothetical modulator that stimulated the elimination of inhibin B was included to further lower the level. All parameters had interindividual variability, and age was a covariate at the E_{max} parameter.

In the development of Corifollitropin alfa extensive modelling has been used,

and the development program has been reported as an example of successful implementation of MBDD [29]. A combination of PKPD population models, statistical regression models, and simulation was used to analyse Corifollitropin alfa trial data and make better informed decisions. It resulted in a dosage regimen of 150 μg and 100 μg for subjects weighing >60 kg and $\leq$60 kg, respectively [150, 152].

6.2 This Research

A particular interest in this PhD project on modelling of FE 999049 in drug development was to investigate the influence of endogenously produced FSH. A high endogenous FSH level at the beginning of treatment could influence the pharmacokinetic characterisation of the drug, add to the variations in exposure, and even more so if the level changes over time. Endogenous FSH should therefore be considered in the modelling.

The standard data analysis method is to baseline correct data when the drug is a naturally occurring substance in the body. It seems unlikely that the endogenous FSH production stay unchanged from the initial baseline value throughout treatment since the ovarian hormones are known to alter the endogenous FSH production and release from the anterior pituitary. Subtracting baseline values from concentrations at all other time points may give lower drug concentrations than they actual are. Hence, using baseline corrected data can potentially induce a bias in the model estimates. If the gonadotropin down-regulation of subjects in clinical trials has been completely successful any interference can be avoided and it might be possible to keep the baseline value steady. The influence of endogenous FSH therefore depends on the type of trial and should be considered according to the study design as well.

Some of the existing models with FSH compounds considered endogenous FSH either as a constant level or that changed over time, but influence from other hormones was not included. All the existing PKPD models listed in Table 6.3 were empirical sequential models, where no feedback to the pharmacokinetics could be incorporated.

A more mechanistic modelling approach may be warranted to account for this hormone dynamics and variations in endogenous FSH both between subjects and over time. In addition, variations in response to treatment can be caused by personal demographics, hormone levels, the great difference in infertility type, and a variety of other factors. As seen in the modelling articles discussed above large variations are observed in both PK concentrations, PD markers, and thus model parameters. Another focus in the model development is to identify possible covariates for explaining some of the variations in drug concentration and effects between subjects.

A full systems pharmacology model including all hormones would be rather complex and require an extensive dataset for proper estimation of all parameters. To have a more focused goal within this PhD framework inhibin B was chosen as the ovarian hormone to incorporate in the model as it has a purely inhibitory effect at FSH. In addition, inhibin B is an important marker in early drug development and is the first hormone to increase upon exogenous FSH stimulation with high correlation to follicular development as discussed in section 4.2.1.

Study				PK Model	
Authors	FSH products	Doses	Subjects	Compartments	Endogenous FSH
le Cotonnec et al. (1993) [92]	Metrodin, Metrodin HP	SD i.v.	12	two	yes
		SD i.m. and s.c.	12*	one	yes
le Cotonnec et al. (1995) [178]	Metrodin	SD i.v.	12	two	yes
		SD i.m.	12	one	yes
le Cotonnec et al. (1994) [179]	Metrodin, Gonal-F	SD i.v.	12	two	yes
le Cotonnec et al. (1994) [180]	Gonal-F	SD i.v.	12	two	yes
		SD i.m. and s.c.	12	one	yes
		MD s.c.	12	one	BLC

Table 6.1: Overview of four studies that have used exponential models to describe the pharmacokinetics of urinary or recombinant FSH in healthy volunteers. SD: single dose, MD: multiple dose, i.v.: intravenously, s.c.: subcutaneous, i.m.: intramuscular. The last two columns indicate how many compartments the PK model had and whether an endogenous FSH supply was included in the model or if data was baseline corrected (BLC). *male subjects.

Study				PK Model		
Authors	FSH products	Doses	Subjects	Compartments	Covariates	Endogenous FSH
Karlsson et al. (1998) [148]	u-hFSH	MD i.m.	62 patients	one	WT at CL	exponential
	rFSH	MD s.c.	60 patients	one	CL_{CR} at bl BMI on k_a	exponential
	rFSH	SD i.v.	12 females	two	ne	linear
	rFSH	SD i.v. and s.c.	12 females	two	ne	linear
	rFSH	SD i.v. and i.m.	12 females	two	ne	linear
	rFSH	MD s.c. and SD i.v.	12 females	two	ne	linear
	u-hFSH	SD i.v.	12 females	two	ne	constant
	u-hFSH	SD i.v. and i.m.	12 females	two	ne	constant
Devroey et al. (2004) [181]	Corifollitropin alfa	SD s.c.	80 patients	one	WT at CL, V	not included
Balen et al. (2004) [182]	Corifollitropin alfa	SD s.c.	55 patients	one	none	not included
The Corifollitropin Alfa Dose-finding Study Group (2008) [149]	Corifollitropin alfa	SD s.c.	234 patients	one	WT at CL, V	not included

Table 6.2: Overview of some studies that have used nonlinear mixed effects modelling to describe the pharmacokinetics of urinary or recombinant FSH in infertile women (patients) or pituitary down-regulated healthy female volunteers (females). The last three columns indicate how many compartments the PK model had, which covariates were identified, and how endogenous FSH was included in the model. SD: single dose, MD: multiple dose, i.v.: intravenously, s.c.: subcutaneous, i.m.: intramuscular. WT: body weight, CL: clearance, CL_{CR}: creatinine clearance, bl: FSH baseline, BMI: body mass index, k_a: absorption rate, V: volume of distribution. ne: not evaluated.

Study	FSH products	Doses	Subjects	PD endpoint	Method	Endogenous FSH	Covariates
Karlsson et al. (1997) [184]	u-hFSH	MD i.m.	62 patients	Follicular growth	NLMEM	no*	yes
	rFSH	MD s.c.	60 patients				
Porchet et al.(1994) [143]	Gonal-F	MD s.c	12 females	inhibin B, E_2	Indirect link	no	no
le Cotonnec et al. (1998)[185]	Gonal-F	MD s.c.	12 females	inhibin B, E_2	Indirect link	yes	no
de Greef et al. (2010) [151]	Corifollitropin alfa	SD s.c.	369 patients	inhibin B	NLMEM	no	yes

Table 6.3: Overview of some studies that have developed PKPD models for urinary or recombinant FSH in infertile women (patients) or pituitary down-regulated healthy female volunteers (females). The PD endpoint and modelling method used are listed, and whether endogenous FSH and covariates were included. SD: single dose, MD: multiple dose, i.v.: intravenously, s.c.: subcutaneous, i.m.: intramuscular. NLMEM: nonlinear mixed effects modelling. *was considered but not added as a term in the model.

Chapter 7

Objectives

The overall aim of the thesis was to develop population PK and PKPD models from clinically observed FE 999049 data using nonlinear mixed effects modelling to acquire a better understanding of the drug's PK and PKPD properties. In addition, the objective was to investigate the influence of endogenous FSH by taking the reproductive endocrine system dynamics into account.

Three specific aims of the analyses were to:

1. Describe the population pharmacokinetics of FE 999049 after single dose administration and examine if part of any interindividual variability in the PK model can be explained by potential person specific covariates (Paper I).
2. Describe the population pharmacokinetics of FE 999049 after multiple dosing and evaluate the influence of endogenous FSH levels on the FE 999049 pharmacokinetics (Paper II).
3. Develop a semi-mechanistic PKPD model describing the relationship between FSH and inhibin B when accounting for variations in endogenous FSH (Paper III).

Chapter 8

Methods

Data from three clinical trials with FE 999049, two phase I studies (CS01 and CS02) and one phase II study (study 000009), were available for the analyses. In this chapter a summary of each clinical study, the generated data, and modelling strategy is given. For further details about the clinical studies the reader is referred to Olsson et al. [186], Olsson et al. [120], and Arce et al. [187], respectively. CS02 and study 000009 included a Chinese hamster ovary (CHO)-derived rFSH marketed product GONAL-F (follitropin alfa, EMD Serono) as an active comparator. In this work, focusing on the PK and PD properties of FE 999049, subjects receiving GONAL-F were excluded. The intention was to develop a PK model based on single dose data from CS01 (paper I). This model structure should overall be confirmed after repeated dosing with the CS02 data (paper II), where additional information could be gained from the different study design and extra hormone measurements. Finally, using the FE 999049 population PK knowledge acquired from phase I data, a simultaneous PKPD model with inhibin B as PD endpoint was developed based on phase II data (paper III).

8.1 Clinical Trials

The trials were performed by Ferring Pharmaceuticals. Each study protocol was approved by independent investigational review boards, regulatory authorities and local ethics committees. The studies were performed according to the Helsinki declaration and good clinical practice. Prior to the studies all participants signed informed consent forms.

Phase I

The phase I studies included healthy female volunteers aged 21-40 years with a normal menstrual cycle, a BMI 18-29 kg/m^2, and using combined monophasic oral contraceptives (COC). Table 8.1 gives an overview of the subjects who received FE 999049 in each trial. In order to avoid any interference with endogenous FSH levels, they were gonadotropin suppressed throughout the study. To verify a proper low endogenous hormone level, serum FSH had to be below 5 IU/L on day -3 and day -1 before dose administration. If not the subjects were excluded.

CS01

CS01 was the first-in-human study with FE 999049. It was a randomised, double-blind, placebo controlled, sequential single ascending dose study investigating the safety, tolerability, and pharmacokinetics of FE 999049. 40 subjects received a single subcutaneous abdominal injection of either 37.5, 75, 150, 225, or 450 IU FE 999049 (equivalent to 2.19, 4.38, 8.76, 13.14, and 26.27 μg, respectively) or placebo. On day -14 before the start of the trial all subjects were switched to the same high-dose COC (OGESTREL 0.5/50), which were taken continuously throughout the study to suppress endogenous FSH.

Blood samples for measurement of serum FSH concentration were collected 60 and 30 minutes prior to administration, immediately before administration, at every 4 hours the first 48 hours and subsequent every day up to 9 days after administration.

CS02

The second clinical study, CS02, was a randomised, double-blind, active control, multiple dose study for investigating the safety, tolerability, immunogenicity, pharmacokinetics and pharmacodynamics of FE 999049. A GnRH agonist (LUPRON DEPOT, 1-month depot) was used to suppress the endogenous FSH levels. Even though it is an agonist i.e. it exerts the same effect as GnRH and stimulates the pituitary gland to secrete gonadotropins, it can be used to suppress the endogenous production of FSH. This is due to the fact that the sensitivity of the GnRH receptors in the pituitary gland decrease under constant exposure to GnRH (agonist) and ultimately the receptors become unresponsive. As a consequence GnRH stimulated gonadotropin secretion ceases. 49 subjects were given daily subcutaneous doses of 225 IU for 7 days of either FE 999049 (dose equivalent to 14.69 μg) or GONAL-F.

Blood samples were collected 60 and 30 minutes prior to administration, immediately before administration, and once a day for 15 days after the first administration. Serum FSH, Inhibin B, estradiol, progesterone, and LH concentrations were measured in the blood samples. In addition, at day 6 after administration of the last dose the FSH concentration was measured every 4th hour for the following 2 days.

Phase II

The 000009 phase IIb dose finding study was a randomised, controlled, assessor-blind, parallel group, multicentre, multiple dose study assessing the dose-response

Study	CS01	CS02	000009
Subjects	30	24	222
Age (years)	28.7 (21-35)	31.1 (21.5-38.7)	32.7 (21-37)
Weight (kg)	64.3 (51.6-90)	71.5 (46.1-86.6)	62.0 (46-95.2)
BMI (kg/m^2)	24.4 (18.7-28.9)	26.6 (20.8-28.9)	22.8 (18.3-32.0)

Table 8.1: Number of subjects in each clinical study who received FE 999049 with the mean value of age, body weight, and body mass index (BMI) followed by range in brackets. Further details about subjects in each trial can be found in the manuscripts paper I, II, and III, respectively, at the end of this thesis.

relationship of FE 999049 in women undergoing an ART programme. 265 women who had been diagnosed with tubal infertility, infertility related to endometriosis stage I/II, unexplained infertility, or have a partner diagnosed with male factor infertility were included. Table 8.1 provides an overview of the personal demographics for the subjects who received FE 999049. Randomisation of patients were stratified according to AMH levels at time of screening: a concentration of 5.0-14.9 pmol/L was defined as low and 15.0-44.9 pmol/L as high AMH level. They received daily subcutaneous doses of either 5.18, 6.90, 8.63, 10.35, or 12.08 μg (equivalent to 90, 120, 150, 180 and 210 IU, respectively) FE 999049 or 11 μg (150 IU) GONAL-F. To prevent a premature LH surge, a GnRH antagonist (0.25 mg ganirelix acetate, ORGALUTRAN, MSD / Schering-Plough) was initiated on stimulation day 5 after the first dose and given daily throughout the stimulation period. Blood samples for FSH, LH, inhibin A, inhibin B, estradiol, progesterone, and testosterone measurements were collected immediately before the first administration, at day 3 and day 5 after the first dose, and hereafter at least every second day. When three follicles $\geq$15 mm were observed, visits and monitoring were performed daily. Doses were given until three or more follicles with a diameter $\geq$17 mm were observed or for a maximum of 16 days. The cycle would be cancelled if there were either too many (more than 35 follicles $\geq$12 mm) or too few (less than three follicles $\geq$10 mm at day 10) growing follicles.

8.1.1 Data

Analysis of serum FSH concentrations from the phase I studies was performed at Ferring Pharmaceuticals A/S, Copenhagen, Denmark, with a validated immunoassay based on electrochemiluminescence (MSD sectorTM Imager 2400) with a lower limit of quantification (LLOQ) of 0.075 μg/L. FSH samples from the Phase II 000009 study were analysed at ICON Central Laboratories, Dublin, Ireland, with a chemiluminescent immunometric assay (IMMULITE 2500 FSH (ROCHE), LLOQ:

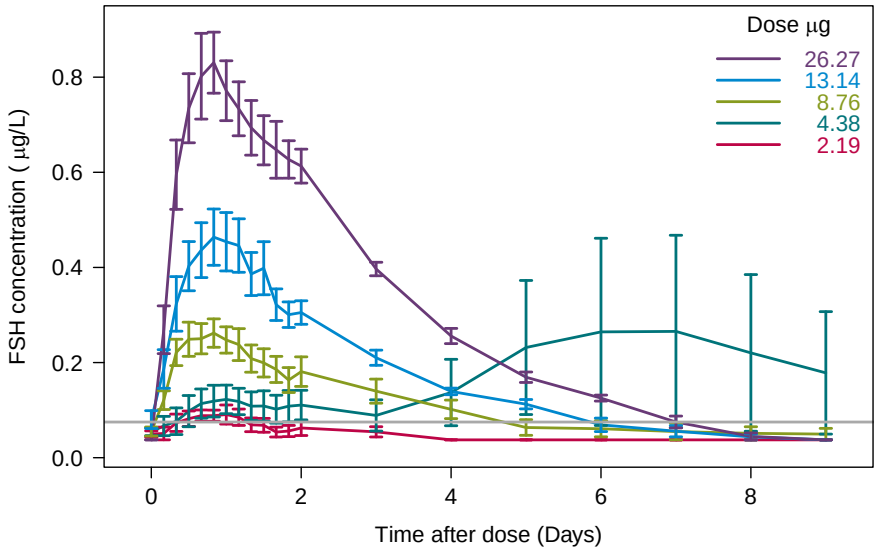

Figure 8.1: The observed serum FSH concentration over time for all subjects in each treatment group in CS01. Lines are mean values with standard error (SE) bars. Observed BQL measurements were set to LLOQ/2 in the plot and the day of administration to day 0. The grey line represents the LLOQ of 0.075 μg/L.

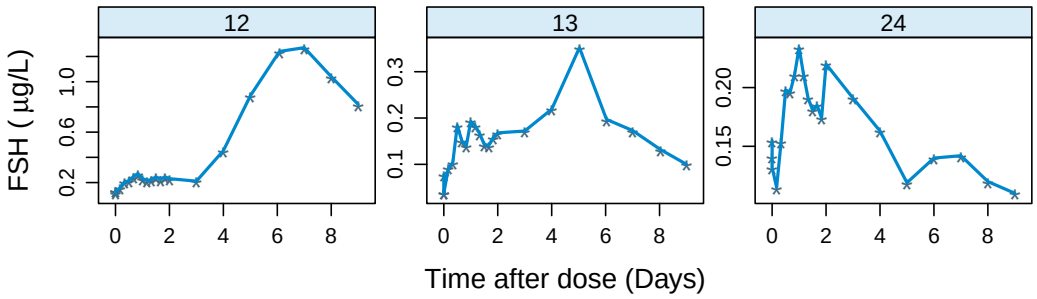

Figure 8.2: The three subjects that are assumed to not have fully suppressed endogenous FSH due to the second increase in observed FSH concentration after day 3. Subject 12 and 13 are from the 4.38 μg dose group and subject 24 is from the 8.76 μg dose group.

0.0052 μg/L). Inhibin B was measured by an enzyme linked immunosorbent assay (Gen II ELISA (Beckman Coulter)) with an LLOQ of 4.8 pg/mL.

To inspect the trend in the datasets and look for any abnormalities, plots for individual concentration-time profiles and mean serum FSH concentrations against time were initially created. Mean of observations for each of the 5 treatment groups in CS01 is shown in Figure 8.1. The 4.38 μg dose group have an odd second increase in FSH concentration starting at day 3 after administration. This pattern was examined further in the individual concentration-time profiles, where two subjects

Study	CS01	CS02	000009
Doses	5	1	5
Dose range (μg)	2.19-26.27	14.69	5.18-12.08
Subjects	27	24	222
Duration (days)	10	16	16
FSH	594	672	1160
FSH BQL	258 (43.4%)	3 (0.4%)	0
Inhibin B	0	383	1155

Table 8.2: Specifications about each of the three modelling datasets. First is given how many FE 999049 dose groups, the dose range, and number of subjects in each dataset. Duration is how many days subjects were monitored in the study. For the 000009 study the number is the maximum number of stimulation days. Number of total FSH and inhibin B measurements in each dataset is listed. For FSH is also given how many of the measurements were below the quantification limit (BQL) with the percentage in brackets.

were identified to cause this increase. In addition, one subject in the 8.76 μg dose group showed a similar tendency (Figure 8.2). A likely explanation for this increase is that their endogenous FSH was not fully suppressed and hereby camouflaging the true rFSH concentration after treatment. In particular the elimination rate of the drug cannot be properly estimated from these subjects. These subjects' concentration-time profiles were considered to not reflect the PK profile for FE 999049 and were thus excluded from the analysis. Table 8.2 gives details about each dataset used for modelling.

8.2 Data Analysis & Modelling

Each of the datasets were analysed separately and described in paper I, paper II, and paper III, respectively, using the population PK(PD) modelling approach with nonlinear mixed effects models. The models were implemented in NONMEM 7.2.0 (Icon Development Solutions, USA) [153] using first order conditional estimation with interaction for model parameter estimation. The statistical program R version 2.11.1 [188] was used for data management, as well as making all graphical representations. VPCs were performed using PsN [189, 190] and plotted using Xpose [191].

8.2.1 Missing Data

The datasets were not balanced since measurements were randomly missing for some subjects. In addition, FSH was measured more frequently than inhibin B in CS02. No action were taken for the single missing values in the modelling estimation process since they were missing at random. When the observed hormone concentrations were needed for testing covariate relationships the missing values were filled out in the following way. A subject without a baseline value were given the median of the population's baselines. For later time points the last observation was carried forward such that a missing value was given the last measured value.

Baseline measurements were taken before dosing, and therefore represent the initial endogenous FSH level. All subjects in the CS01 study had a BQL measurement as FSH baseline value, meaning that their endogenous FSH level was not measurable. Whereas CS02 subjects had higher and measurable baseline values. Due to the different extent of FSH BQL measurements in CS01 and CS02 data they were handled differently in the model development. In paper I the M3 method [171] was used since over 40% of the CS01 data were BQL. With only three BQL points in the CS02 data, it was chosen to exclude them (M1 method [171]) for the model development in paper II.

8.2.2 Modelling Strategy

For the population PK structural model, one- and two-compartment distribution models were tested. A potential delay in the absorption process was examined by transit compartments and lag time. It was checked if an endogenous FSH contribution to the total FSH concentration could be identified, if the endogenous level changed during the course of the trial, and if it influenced the results.

A combined additive and proportional error model was used for the residual error. If one of the parameters was too small or poorly estimated a reduced error model was tested. Variations in exposure between subjects were established by adding random effects (IIV) to the parameters, subsequently any correlations between the identified random effects were examined.

The parameters and their random effects were plotted against potential covariates to investigate whether some of the IIV could be explained. If a trend was seen in the plots, the parameter-covariate relationship was tested in the model. After adding covariates it was checked if any of the IIV random effect parameters could be removed. Covariates available in the CS01 data were limited to body weight, age, and height. In CS02 there were additionally measured LH, progesterone, estradiol, and inhibin B hormone levels. The baseline values for the latter three hormones, that are produced by the ovaries and affect the FSH production by feedback mechanisms, were tested as covariates at the baseline FSH. Inhibin B

was also tested as a time-varying covariate at the endogenous FSH level using the longitudinal data. To get comparable population parameters between studies 65kg was chosen as the normalisation factor when testing body weight as a covariate since it was an average standard weight.

First the structural model was developed, then the random effects matrix identified, and lastly covariates were added. However, the sub-models are intertwined i.e. the choice of one sub-model affects the others, therefore, continuously during the model development several iterations of checking the significance of earlier added or discarded model components were performed.

8.2.3 Pharmacokinetic-Pharmacodynamic Analysis

In the 000009 study PK data was sparse with a limited number of samples collected. Therefore, k_a and V were fixed to the values from paper II in the PKPD model (paper III). The focus in paper III was to describe the exposure-response relationship for FSH and inhibin B taking the hormone dynamics into account. A simultaneous modelling approach was therefore used. Generally, the same modelling principles used for the PK models were applied in the PKPD model. With the extension of linking the PK model to the inhibin B concentration, the modelling becomes more complicated with additional types of interactions between the hormones and relations in the model to explore. The FSH stimulation of inhibin B was tried to be modelled as both an indirect and an indirect delayed effect with different functions describing the effect. Likewise for the inhibin B inhibition of endogenous FSH.

A turnover model, where no change in concentrations at time zero is assumed when the equations fulfil the initial condition, was used for the FSH and inhibin B concentrations. The intention of administering the GnRH antagonist was to avoid a premature LH surge, but this also inhibits the production of FSH. To follow the protocol this inhibition of endogenous FSH after day 5 should be included in the model.

In addition to the covariates tested in the other models, any influence of AMH level was examined. Data was log-transformed and separate error models for FSH and inhibin B was used.

8.2.4 Evaluation Criteria

Model development was guided by changes in the OFV, and graphical model assessments by goodness-of-fit plots and VPCs. A significance level of 0.05 was used for the χ^2-test to discriminate between nested models. For two non-nested models with equal number of parameters the model with lowest OFV was chosen. In

addition, the precision of parameter estimates expressed as relative standard error (RSE) were considered as well as keeping in mind if the parameter value was realistic. For testing covariates the significance was also evaluated by the change in %CV, i.e. how much the unexplained IIV decreased from adding the covariate.

Chapter 9

Results

The results of the data analyses and modelling are described in the three manuscripts, one for each of the three aims of this PhD thesis. Paper I and paper II presents the PK model developed from the phase I CS01 and CS02 data, respectively. Paper III describes the PKPD model that relates the FSH concentration to the inhibin B PD response in the phase II 000009 study.

9.1 Pharmacokinetic Properties of FE 999049

There were some differences between the two phase I PK studies in the observed FSH levels at baseline, hence the datasets were not integrated but two separate PK models were developed in paper I and paper II with two alternative methods for handling BQL data. In paper I the FE 999049 PK was described following a single dose (CS01 data). In paper 2 the PK was described following multiple administration (CS02 data).

9.1.1 Pharmacokinetic Model (Paper I)

From the CS01 data the best structural model was found to be a one-compartment distribution model with first order absorption. In order to adequately describe the absorption phase a one compartment transit model was introduced to add a time delay in the absorption from the dosing site to the central compartment. The PK model is described by the differential equations (9.1)-(9.3).

$$\frac{d\text{rFSH}_{DS}(t)}{dt} = -k_{tr}\text{rFSH}_{DS}(t) \quad (9.1)$$

$$\frac{d\text{rFSH}_{TR}(t)}{dt} = k_{tr}\text{rFSH}_{DS}(t) - k_a\text{rFSH}_{TR}(t) \quad (9.2)$$

$$\frac{d\text{rFSH}(t)}{dt} = k_a\text{rFSH}_{TR}(t) - k\,\text{rFSH}(t). \quad (9.3)$$

$\text{rFSH}_{DS}(t)$, $\text{rFSH}_{TR}(t)$, and $\text{rFSH}(t)$ is the FE 999049 amount left at the dosing site, in the transit compartment, and in the central compartment at time t, respectively. The pre-dose amount in the central and transit compartment is zero, but the dosing site is initiated by the amount of dose given. Since data was obtained

after subcutaneous dosing, the parameters estimated were CL/F and V/F. To obtain the predicted serum concentration in the central compartment the amount rFSH(t) is divided by V/F. The absorption rate from the dosing site into the transit compartment is k_{tr}. FE 999049 is absorbed to the central compartment with rate k_a, from where it is eliminated with rate k, which is given by CL/V . An endogenous FSH supply to the central compartment could not be identified by the model, and the measured FSH serum concentrations could therefore be regarded as FE 999049 with an insignificant amount of endogenous FSH. An IIV was detected at CL/F, V/F, and k_a, and the variances for the IIV random effect parameters on CL/F and V/F are positively correlated. A combined additive and proportional error model was necessary to describe the residual error.

No influence of dose at the parameters were found, indicating that the pharmacokinetics is linear. The only covariate identified with statistical significance was body weight allometrically scaled at CL/F and V/F. A standard weight of 65 kg was chosen as normalisation factor for the covariate effect. The importance of body weight being a factor influencing the parameters was further supported by a reduction in the unexplained IIV from 31.5 to 28.2% CV for CL/F and from 46.4 to 44.3% CV for V/F upon inclusion of body weight in the model. Figure 9.1 illustrates how CL/F and its random effect varied with weight. Adding body weight as a covariate do not change the individual parameters but the trend in the random effects with weight is gone, implying that the relationship is described properly.

Final model parameters are listed in Table 9.1. The graph with mean observed data and typical model predictions for each dose (Figure 9.2) together with the diagnostic plots (Figure 9.3 - 9.4) indicate the model describes data well. Conditional weighted residuals cannot be calculated when the M3 method is used, because BQL data points do not provide a prediction but a probability. Instead, individual weighted residuals was used for the diagnostic plots. A two panel VPC illustrating both continuous and categorical (BQL) data was used [173]. For this model the 2.5th percentile of observations is not shown since it solely consists of BQL points and therefore falls outside the plotting area for the top panel.

Using the PK model based on CS01 single dose data, the expected time-course of FE 999049 following multiple dose administration was simulated for three women with different body weights to illustrate the impact of body weight on FSH exposure. The resulting PK profiles in Figure 9.5 vary substantially for the three individuals. The inclusion of body weight at CL/F and V/F clearly makes a difference in the model predicted drug concentration. Through simulations of different doses it was found that on average a 100 kg woman would need doses of 18 μg to get a similar exposure as a 50 kg woman receiving 10 μg doses (Figure 9.6).

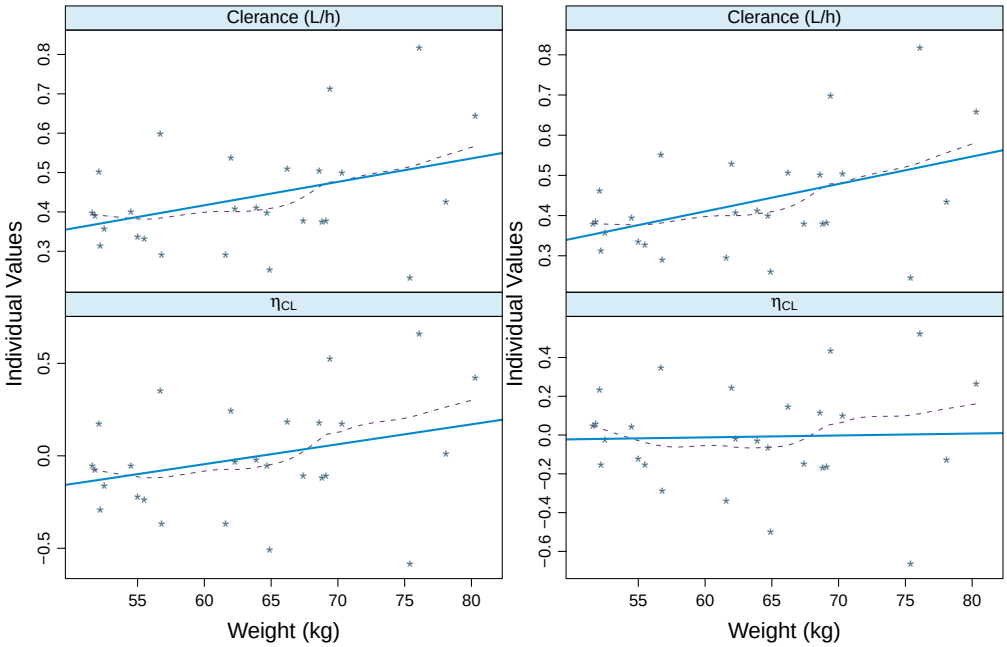

Figure 9.1: Illustration of covariate effect of body weight in the first model from the CS01 data. Individual clearance (CL) values and respective individual random effects (η_{CL}) against body weight (points) in top and bottom panels, respectively, before (left) and after (right) body weight was added as a covariate. The relationship is illustrated with a smooth lowess line (broken purple) and a linear regression line (blue).

Parameter	Estimate	(RSE%)	IIV CV%	(RSE%)
CL/F (L/h)	0.43	(6.3)	28.2	(29.5)
V/F (L)	28.0	(9.1)	44.3	(20.7)
k_{tr} (h^{-1})	0.517	(24.8)		
k_a (h^{-1})	0.16	(12.9)	23.3	(27.7)

Table 9.1: Typical population pharmacokinetic parameter estimates obtained from modelling of CS01 data (paper I) with the relative standard error (RSE) in brackets. For CL/F and V/F the value is the typical value for a woman weighing 65 kg. The interindividual variability (IIV) is listed as the percentage coefficient of variation (CV) with RSE in brackets. F: bioavailability, CL/F: apparent clearance, V/F: apparent volume of distribution, k_{tr}: absorption rate from the dosing site, k_a: absorption rate to the central compartment.

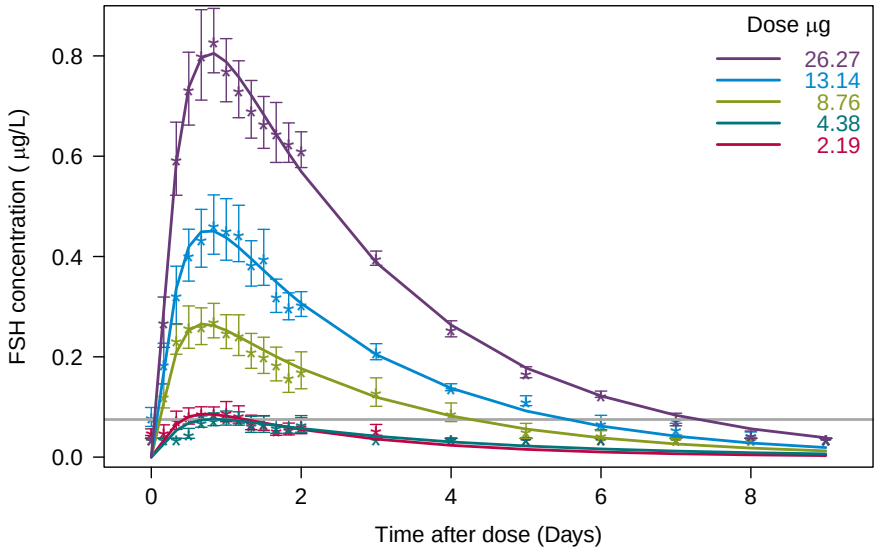

Figure 9.2: Illustration of observed FSH concentrations and model predictions for each treatment group from the model based on CS01 data. Points are mean of observations with standard error (SE) bars. Observed BQL measurements were set to LLOQ/2 in the plot. Lines are typical model prediction. The grey line represents the LLOQ of 0.075 μg/L.

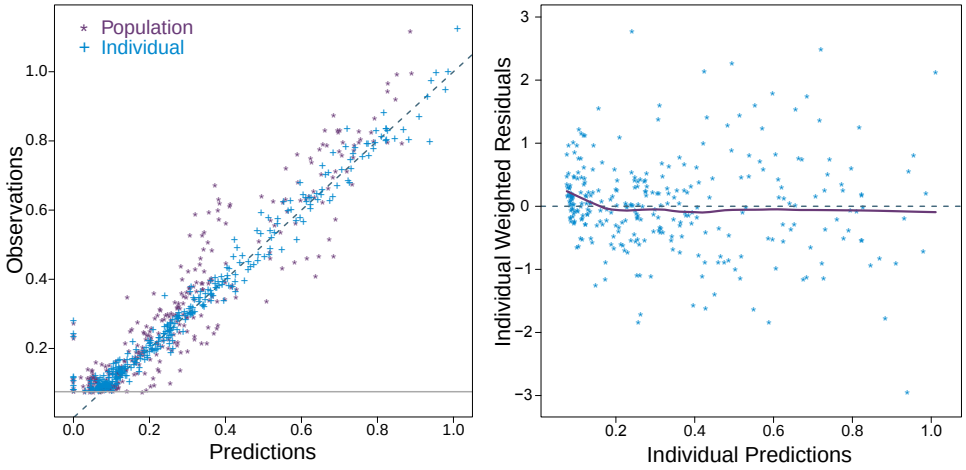

Figure 9.3: Goodness of fit plots for the model based on CS01 data. Left: Observations against population predictions (purple points *) and individual predictions (blue points +) with the unity line. The grey line represents the LLOQ of 0.075 μg/L. Right: Individual residuals against individual predictions (points) with a smooth lowess line.

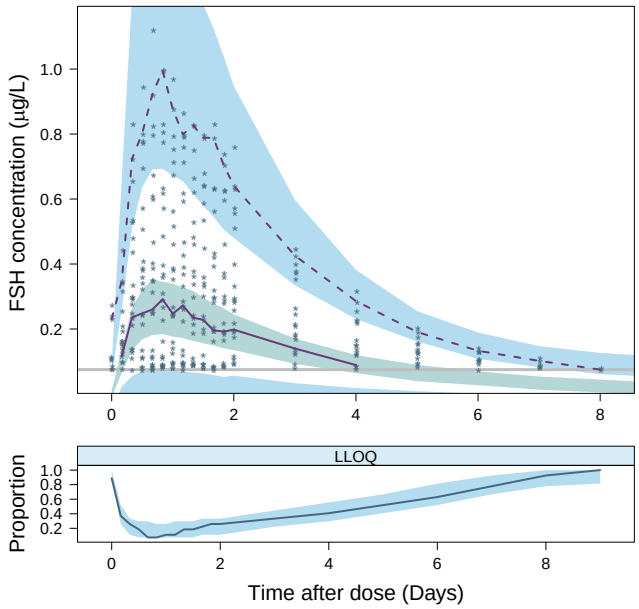

Figure 9.4: Two panel visual predictive check for the CS01 model. The top panel shows the observations above LLOQ (points) and the 50th and 97.5th percentiles of observations (purple lines). The 2.5th percentile of observations is not shown since it solely consists of BQL points. The shaded areas are the simulated 95% confidence intervals (CI) for the 2.5th, 50th, and 97.5th percentiles. The grey line represents the LLOQ of 0.075 μg/L. In the bottom panel the blue line is the fraction of BQL observations with the 95% CI for the median from simulations.

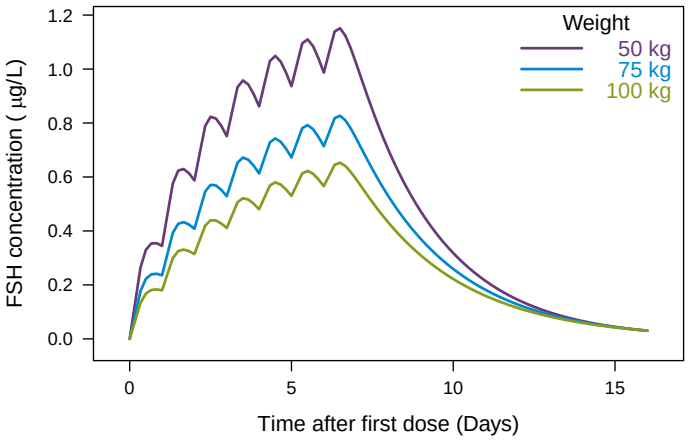

Figure 9.5: Simulations of body weight effect based on the model from CS01 single dose data. The expected FSH concentration simulated after multiple dosing of 10 μg FE 999049 for three subjects with different body weights.

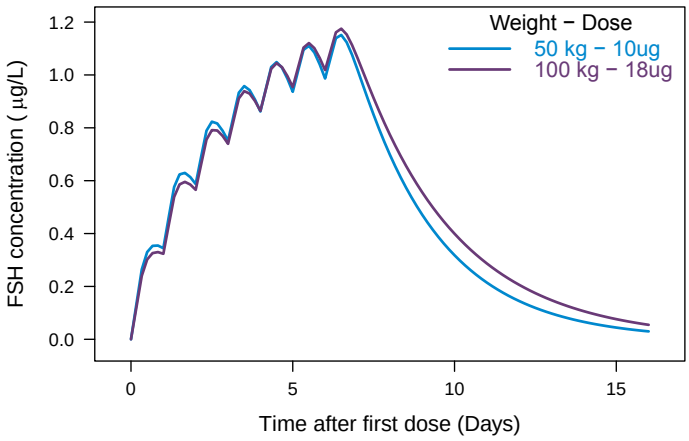

Figure 9.6: Simulations based on the model from CS01 single dose data of required dose to get same exposure in two different women. Simulation results of the expected FSH concentration after multiple dosing of 10 μg FE 999049 to a woman weighing 50 kg (blue line) and 18 μg to a woman weighing 100 kg (purple line).

9.1.2 Modelling Endogenous FSH Levels (Paper II)

The PK model developed from the CS01 data could not entirely be used as a starting point for paper II, since there were measurable endogenous FSH concentrations at baseline (FSH_{bl}) before dosing in the CS02 data that would have to be accounted for. Additionally, there was no need for using the M3 method for the CS02 data. Therefore, initially a reduced model in form of a one-compartment distribution PK model with first order absorption and a combined additive and proportional error model was used as starting model. An endogenous FSH supply had to be added at first to correct for a clear under-prediction, especially at baseline. The total FSH amount at time t in the central compartment was therefore a sum of the endogenous FSH and the exogenously administered rFSH:

$$\mathrm{FSH}(t) \quad = \quad \mathrm{FSH}_{\mathrm{en}}(t) + \mathrm{rFSH}(t). \tag{9.4}$$

The endogenous FSH was included in the model as a contribution to the differential equation for the central compartment with zero order production rate constant k_{endo} and was assumed to have the same elimination rate constant as FE 999049. A random effect for IIV was found to be significant at CL/F, V/F, k_{tr}, and FSH_{bl}. CL/F and V/F were positively correlated, and body weight normalised to 65 kg was an allometrically scaled covariate at CL/F and V/F with statistical significance. Adding a transit compartment at this stage was not significant.

The individual estimated FSH_{bl} values were evaluated against observed baseline values of estradiol, inhibin B, and progesterone (Figure 9.7). Of these proges-

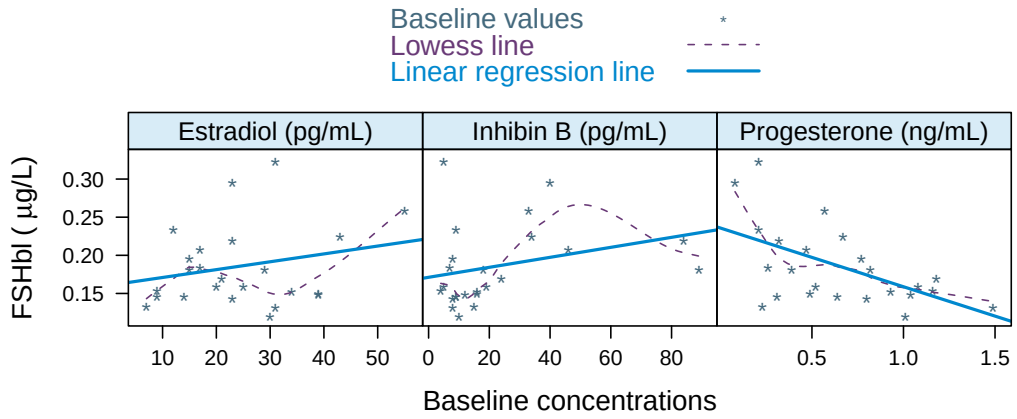

Figure 9.7: Relationship in the CS02 model and data between individual estimated endogenous baseline values $\mathrm{FSH_{bl}}$ and observed estradiol, inhibin B, and progesterone baseline values (points), respectively. Illustrated with a smooth lowess line (broken purple) and a linear regression line (blue).

terone baseline was the only significant baseline relationship in the model when included as a negative correlation with $\mathrm{FSH_{bl}}$. A linear, exponential, $\mathrm{I_{max}}$, and power function was tested for describing the covariate relationship. Of these a power function was most significant (Figure 9.8). Furthermore, observed inhibin B levels (InhB(t)) was a time-varying inhibitory covariate at the endogenous FSH production and was best described by an $\mathrm{I_{max}}$ function (see equation (9.5)). Figure 9.9 illustrates how the inhibin B concentration suppress the model predicted endogenous FSH concentration over time. It was re-tested if a transit compartment could be added in the absorption process and it was significant better after the covariate relations had been added. The overall model structure from first in human data was thus confirmed. Accordingly, the PK model based on CS02 data consists of equation (9.1) and (9.2) from the first PK model for the absorption process, and in order to incorporate the study design differences the change in FSH amount in the central compartment is described by

$$\frac{d\mathrm{FSH}(t)}{dt} = k_{\mathrm{endo}}\left(1 - \frac{\mathrm{InhB}(t)}{\mathrm{InhB}(t) + \mathrm{IC_{50}}}\right) + k_a \mathrm{rFSH}_{TR}(t) - k\,\mathrm{FSH}(t). \quad (9.5)$$

Values of $\mathrm{FSH_{bl}}$ was estimated for each subject to be the initial concentration in the central compartment before dosing, thus the initial condition for equation (9.5) in amount is $\mathrm{FSH_{bl}}$*V/F. The parameter $\mathrm{IC_{50}}$ is the concentration yielding half of maximum inhibin B suppression. It was assumed that at time zero the change in FSH amount is zero as a turnover model and consequently k_{endo} is given by the other parameters at pre-dose values.

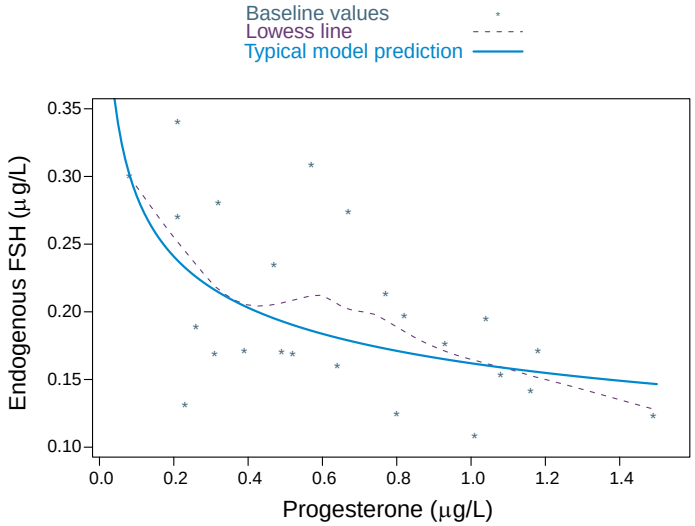

Figure 9.8: Individual estimated endogenous baseline values FSH_{bl} from the CS02 model against observed progesterone baseline values (points) with a smooth lowess line (broken purple line). The blue solid line is the typical population relationship described by a power function.

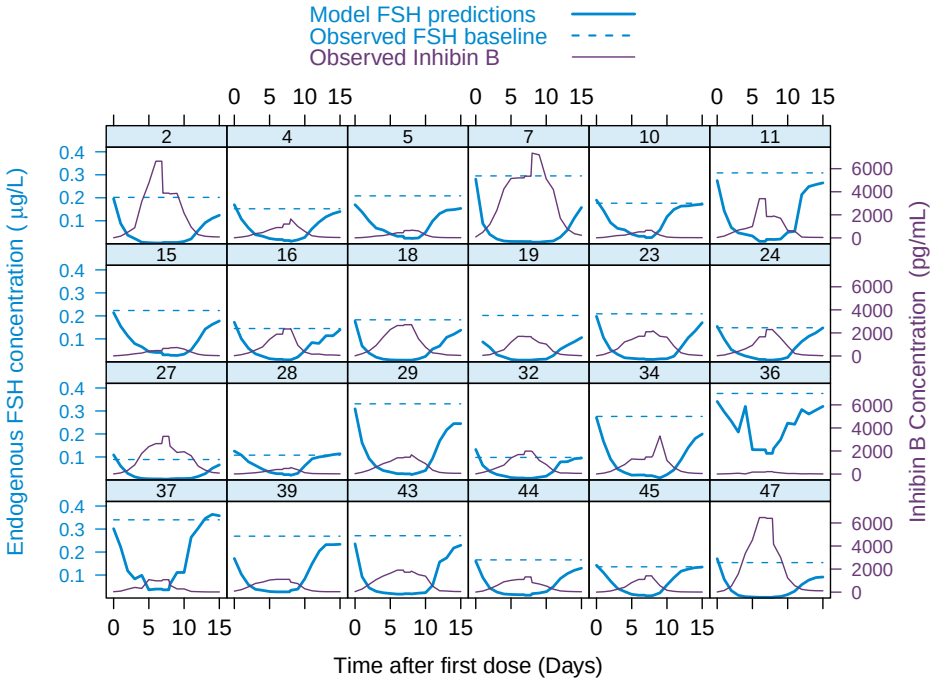

Figure 9.9: Individual hormone concentration profiles over time for subjects in CS02. The broken blue line is the observed endogenous FSH baseline level when assuming it is constant throughout the trial. The solid blue line is the model predicted endogenous FSH level obtained with inhibition by the observed inhibin B levels (purple line) over time. The number at each subplot is the subject ID number.

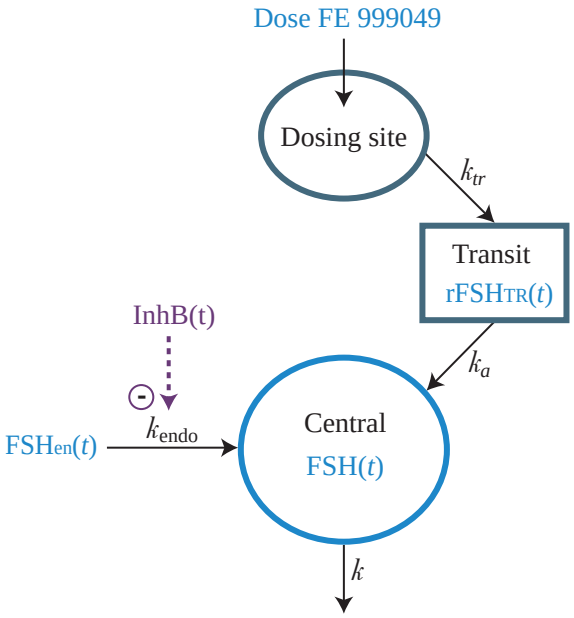

Figure 9.10: Compartment diagram of the PK model from the CS02 data. Contributions to the total FSH amount in the central compartment ($\mathrm{FSH}(t)$) are FE 999049 from the transit compartment ($\mathrm{rFSH}_{\mathrm{TR}}(t)$) and endogenous FSH ($\mathrm{FSH}_{\mathrm{en}}(t)$). The endogenous FSH production rate (k_{endo}) is inhibited by inhibin B concentrations ($\mathrm{InhB}(t)$). k_{tr}: absorption rate from the dosing site, k_a: absorption rate to the central compartment, k: elimination rate.

The model is illustrated by the compartment diagram in Figure 9.10 and the parameters are listed in Table 9.2. The VPC in Figure 9.11 indicates that the model and estimated parameters adequately describe data.

The final model was re-evaluated to see if any covariate relationships or correlations had become excessive after others had been added. A significant increase in OFV as a result of removing any of the effects revealed that the model could not be reduced (Table 9.3). Moreover, removing body weight as a covariate increased the unexplained IIV from 15.6 to 18.1% CV for CL/F and from 18.4 to 22.0% CV for V/F. Not including inhibin B as a covariate in the final model resulted in an increase from 83.4 to 164.9% CV at k_{tr}. IIV at $\mathrm{FSH}_{\mathrm{bl}}$ increased from 27.8 to 32.6% CV when the progesterone effect was removed.

Using the model based on CS02 multiple dose data the effect of having body weight as a covariate was evaluated by simulating the expected FSH exposure for three subjects with different weights. In the simulation the same observed inhibin B and progesterone values were used for all three subjects and simulations were performed with frequent time points to get the full dosing profile after 7 daily doses of 10 μg FE 999049. The simulated PK profiles follow the relationship of lower exposure with higher body weight (Figure 9.12).

Parameter	Estimate	(RSE%)	IIV CV%	(RSE%)
CL/F (L/h)	0.423	(3.9)	15.6	(12.7)
V/F (L)	24.3	(4.6)	18.4	(14.4)
k_{tr} (h^{-1})	0.329	(17.0)	83.4	(24.1)
k_a (h^{-1})	0.148	(13.2)		
FSH_{bl} (μg/L)	0.162	(9.1)	27.8	(16.5)
$Progbl_{ef}$	-0.246	(34.7)		
IC_{50} (pg/mL)	100.0	(37.2)		

Table 9.2: Typical population parameter estimates obtained from modelling of the CS02 data (paper II) with the relative standard error (RSE) in brackets. For CL/F and V/F the value is the typical value for a woman weighing 65 kg. The interindividual variability (IIV) is listed as the percentage coefficient of variation (CV) with RSE in brackets. F: bioavailability, CL/F: apparent clearance, V/F: apparent volume of distribution, k_{tr}: absorption rate from the dosing site, k_a: absorption rate to the central compartment, FSH_{bl}: endogenous FSH baseline, $Progbl_{ef}$: power exponent for progesterone baseline covariate effect, IC_{50}: inhibin B concentration yielding half suppression.

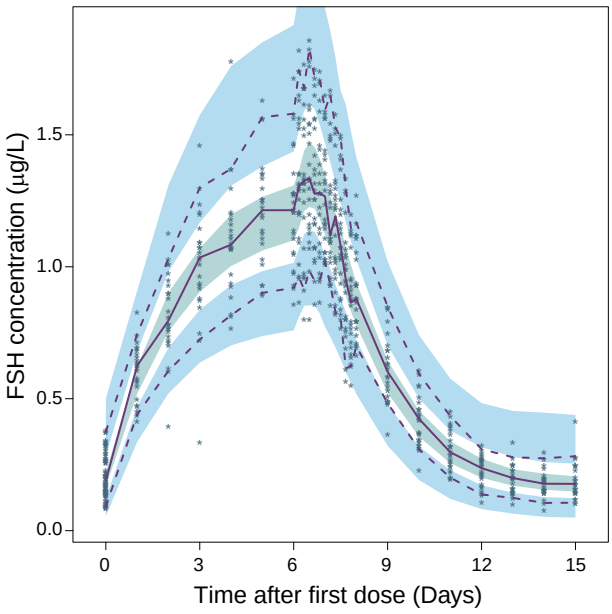

Figure 9.11: Visual predictive check for the CS02 model showing the FSH observations (points) and the 2.5th, 50th, and 97.5th percentiles of observations (purple lines). The shaded areas are the simulated 95% confidence intervals (CI) for the 2.5th, 50th, and 97.5th percentiles.

Removing	df	dOFV
WT at CL/F	0	12.61
WT at V/F	0	14.36
WT at CL/F and V/F	0	8.91
cov(CL/F, V/F)	1	11.77
Progesterone effect	1	7.01
Inhibin B effect	1	78.01
Removing η at		
CL/F	2	399.63
V/F	2	48.69
k_{tr}	1	10.43
FSH_{bl}	1	104.93

Table 9.3: The resulting increase in objective function value (dOFV) when removing covariates, random effect η's for IIV, or the correlation between CL/F and V/F (cov(CL/F,V/F)). When removing η at either CL/F or V/F it is also necessary to remove the correlation, therefore is df = 2. WT: body weight, df: degrees of freedom, F: bioavailability, CL/F: apparent clearance, V/F: apparent volume of distribution, k_{tr}: absorption rate from the dosing site, FSH_{bl}: endogenous FSH baseline.

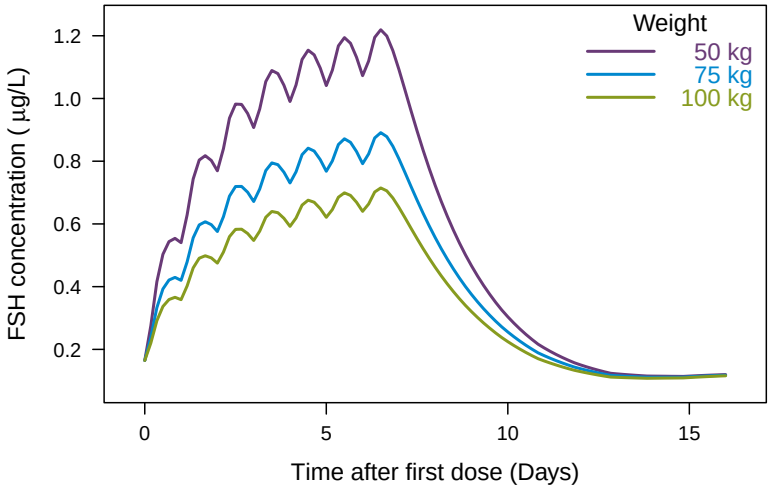

Figure 9.12: Illustration of body weight effect on FSH exposure using the model from CS02 multiple dose data. The FSH concentration-time profiles are obtained from simulations of three subjects with different body weights receiving multiple dosing of 10 μg FE 999049.

9.2 Semi-Mechanistic Dose-Concentration-Response Model (Paper III)

A simultaneous dose-concentration-response PKPD model with inhibin B as PD endpoint was developed from the phase II 000009 data. An indirect response turnover model was used to describe the inhibin B response upon FSH stimulation. The PKPD relationship was modelled simultaneously in order to include a negative feedback of inhibin B concentration at the endogenous FSH production rate.

The PK model from paper II without hormone covariates was used as a starting point. In the 000009 data the PK information was very sparse and not enough to estimate all PK parameters, so k_a and V/F were fixed to values from paper II. After re-testing the PK structure in the simultaneous PKPD model the PK part was the one from paper II with a few alterations.

An I_{max} function was most significant in describing the inhibition of endogenous FSH by the predicted inhibin B concentrations as opposed to using the observed as in paper II. The GnRH antagonist given to suppress the LH secretion from day 5 also affects the endogenous FSH. Therefore, an inhibitory effect at k_{endo} from the antagonist had to be added after day 5 for the model to be in accordance with the trial design. This meant two types of suppression at the endogenous FSH which caused estimation problems. It was tested if the suppression was total after day 5 or if one of the effects took over, but both were significant. So IC_{50} for the inhibin B inhibitory effect on endogenous FSH was fixed to the value obtained in paper II for a more stable model.

A power function with exponent λ for the FSH stimulation of the inhibin B production rate k_{in} was found to describe data best. The power function was given by

$$\mathrm{FSH}_{\mathrm{stim}} = \left(\frac{\mathrm{FSH}(t)}{\mathrm{V/F} \cdot \mathrm{FSH}_{\mathrm{bl}}} \right)^{\lambda}, \tag{9.6}$$

where the total FSH amount ($\mathrm{FSH}(t)$) is divided by V/F to achieve concentrations and it is normalised with endogenous FSH baseline concentrations.

For both FSH stimulation and inhibin B inhibition linear and exponential relationships were tested but they either gave significant worse OFV, a worse fit with parameters poorly estimated, or the model became unstable. It was neither possible having an E_{max} model describing the FSH stimulation because the EC_{50} and E_{max} parameters varied greatly and was estimated poorly with high RSE. There were indications of a delayed response but data did not support estimation of an effect compartment.

The model compartment diagram is illustrated in Figure 9.13 and the corresponding differential equations for the model are

$$\frac{d\text{rFSH}_{DS}(t)}{dt} = -k_{tr}\text{rFSH}_{DS}(t) \tag{9.7}$$

$$\frac{d\text{rFSH}_{TR}(t)}{dt} = k_{tr}\text{rFSH}_{DS}(t) - k_a\text{rFSH}_{TR}(t) \tag{9.8}$$

$$\begin{aligned}\frac{d\text{FSH}(t)}{dt} &= k_{\text{endo}}(1-\text{ANTA}_{\text{ef}})\left(1-\frac{\text{InhB}(t)}{\text{InhB}(t)+\text{IC}_{50}}\right)+k_a\text{rFSH}_{TR}(t)\\ &\quad - k\,\text{FSH}(t)\end{aligned} \tag{9.9}$$

$$\frac{d\text{InhB}(t)}{dt} = k_{\text{in}}\left(\frac{\text{FSH}(t)}{\text{V/F}\cdot\text{FSH}_{\text{bl}}}\right)^{\lambda} - k_{\text{out}}\text{InhB}(t), \tag{9.10}$$

where ANTA_{ef} is the suppressive effect of the GnRH antagonist administered after day 5 and is given by

$$\text{ANTA}_{\text{ef}} = \begin{cases} 0 & , \quad \text{t} \leq 5 \\ \dfrac{\text{GnRH}_{\text{anta}}}{1+\text{GnRH}_{\text{anta}}} & , \quad t > 5 \end{cases} \tag{9.11}$$

Individual inhibin B baseline values (InhB_{bl}) were estimated as initial conditions for equation (9.10), since pre-dose inhibin B concentrations were present. Initial conditions for equation (9.9) were estimated individual endogenous FSH baseline concentrations (FSH_{bl}) multiplied by V/F to get FSH amount. The elimination rate constant k_{out} for inhibin B varied greatly with poor estimation and was therefore fixed. Since it was a turnover model the inhibin B production rate k_{in} was given by the other parameters at initial conditions.

The parameters CL/F, k_{tr}, FSH_{bl}, InhB_{bl}, and λ varied between subjects and an IIV random effect was added at these parameters. An IIV could not be identified for the fixed V/F parameter, but body weight was still tested and found to be a statistically significant covariate at V/F. Furthermore, body weight was a covariate at CL/F, k_{tr}, and λ with a reduction in the variation from 27.2, 51.1, and 48.9% CV to 23.1, 48.3, and 42.7% CV, respectively. A full Ω covariance matrix was significant better and all correlations were well estimated with no extreme values. Separate combined additive and proportional error models were added for FSH and inhibin B.

The PKPD model parameters are given in Table 9.4. The mean predictions fit observations nicely (Figure 9.14) but in the VPC (Figure 9.15) an over-prediction for the lowest dose and an under-prediction for the highest dose is observed for

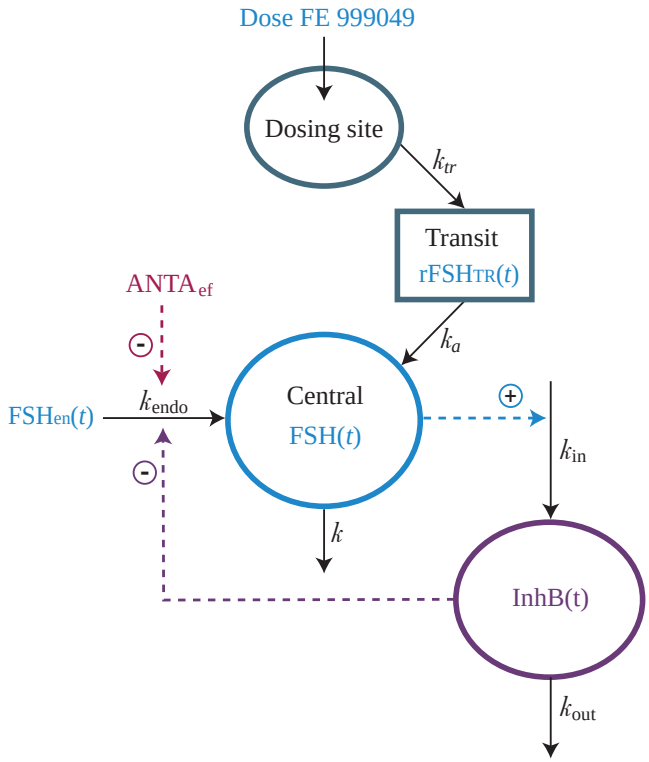

Figure 9.13: Compartment diagram of the PKPD model in paper III. Contributions to the total FSH amount in the central compartment (FSH(t)) are FE 999049 from the transit compartment (rFSH$_{\mathrm{TR}}$(t)) and endogenous FSH (FSH$_{\mathrm{en}}$(t)). The endogenous FSH production rate (k_{endo}) is inhibited by predicted inhibin B concentrations (InhB(t)) and after day 5 also by a gonadotropin releasing hormone (GnRH) antagonist. The inhibin B production rate (k_{in}) is stimulated by FSH. k_{tr}: rFSH absorption rate from the dosing site, k_a: rFSH absorption rate to the central compartment, k: FSH elimination rate from the central compartment, k_{out} inhibin B elimination rate.

Parameter	Estimate	(RSE%)	IIV CV%	(RSE%)
CL/F (L/h)	0.601	(2.2)	23.1	(7.4)
V/F (L)	24.3*			
k_{tr} (h^{-1})	0.0163	(5.0)	48.3	(11.4)
k_a (h^{-1})	0.148*			
FSH_{bl} (μg/L)	0.355	(2.4)	25.3	(5.3)
$InhB_{bl}$ (pg/mL)	79.8	(4.5)	35.9	(10.3)
IC_{50}	100.0*			
$GnRH_{anta}$	0.434	(14.7)		
k_{out}	0.7†			
λ	4.16	(4.6)	42.7	(10.5)
Power exponent for body weight at				
CL/F	0.75†			
V/F	1†			
k_{tr}	-1.40	(26.4)		
λ	1.43	(19.6)		

Table 9.4: Typical population parameter estimates obtained from modelling of the 000009 data (paper III) with the relative standard error (RSE) in brackets. For CL/F, V/F, k_{tr}, and λ the value is the typical value for a woman weighing 65 kg. The interindividual variability (IIV) is listed as the percentage coefficient of variation (CV) with RSE in brackets. F: bioavailability, CL/F: apparent clearance, V/F: apparent volume of distribution, k_{tr}: absorption rate from the dosing site, k_a: absorption rate to the central compartment, FSH_{bl}: endogenous FSH baseline, $InhB_{bl}$: inhibin B baseline, IC_{50}: inhibin B concentration yielding half of maximum suppression, $GnRH_{anta}$: suppressive effect of the gonadotropin releasing hormone (GnRH) antagonist, k_{out}: inhibin B elimination rate constant, λ: the power exponent for the FSH stimulation at inhibin B. * fixed to values from paper II. † parameter fixed value.

FSH concentrations and inhibin B is over-predicted for the highest dose. Since the frequency subjects come in to the clinic for measurements depends on fulfilment of pre-set criteria for follicle number and size, different number of subjects are measured per day. There are even days with only one subject and therefore the observed percentiles in the VPC collapse to the same value. A log-linear relationship was tested for the FSH stimulation instead of a power function. It improved the VPCs for inhibin B by reducing the over-prediction, but the OFV increased by 100.

Simulations of FSH and inhibin B concentrations after multiple dosing in patients of different body weights were performed using the simultaneous PKPD

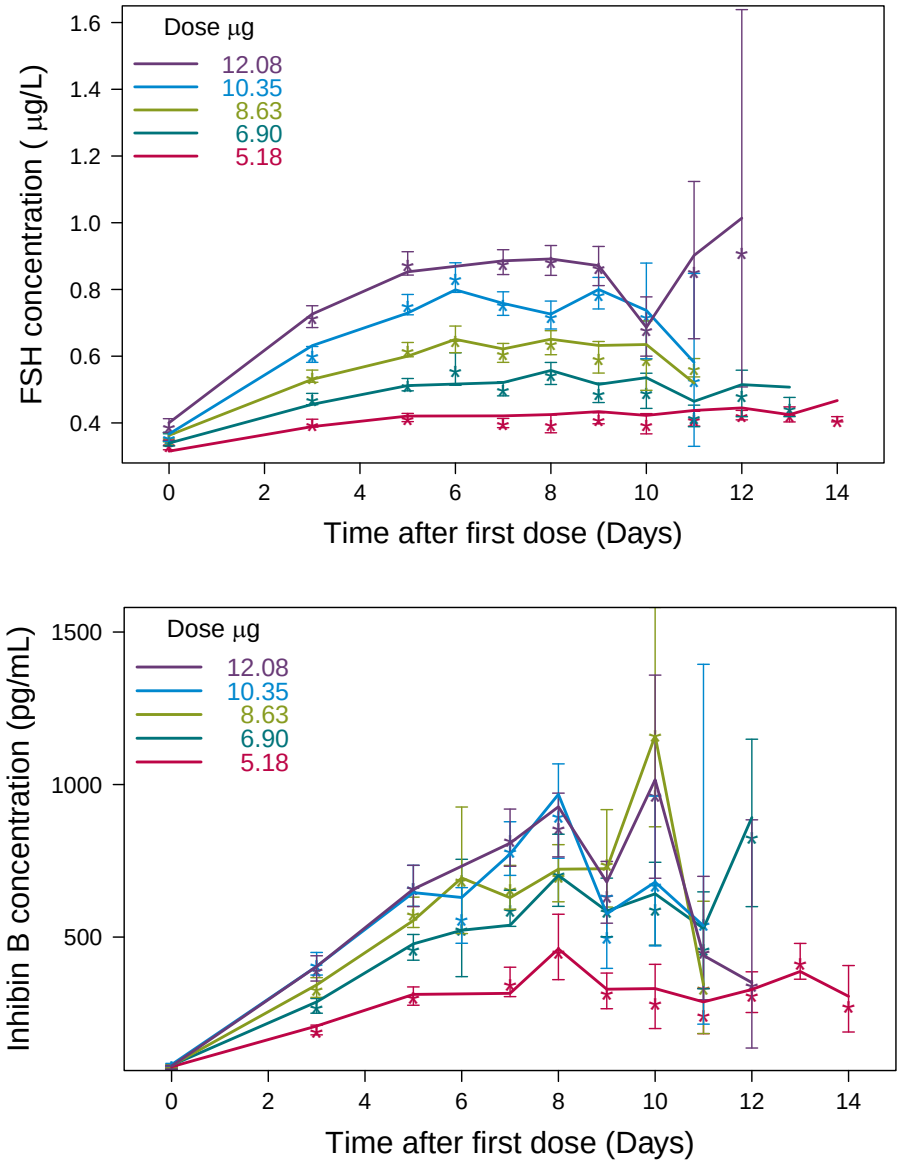

Figure 9.14: Illustration of observed FSH (top) and inhibin B (bottom) concentrations and model predictions for each treatment group in the 000009 study. Points are mean of observations with standard error (SE) bars. Lines are typical model predictions.

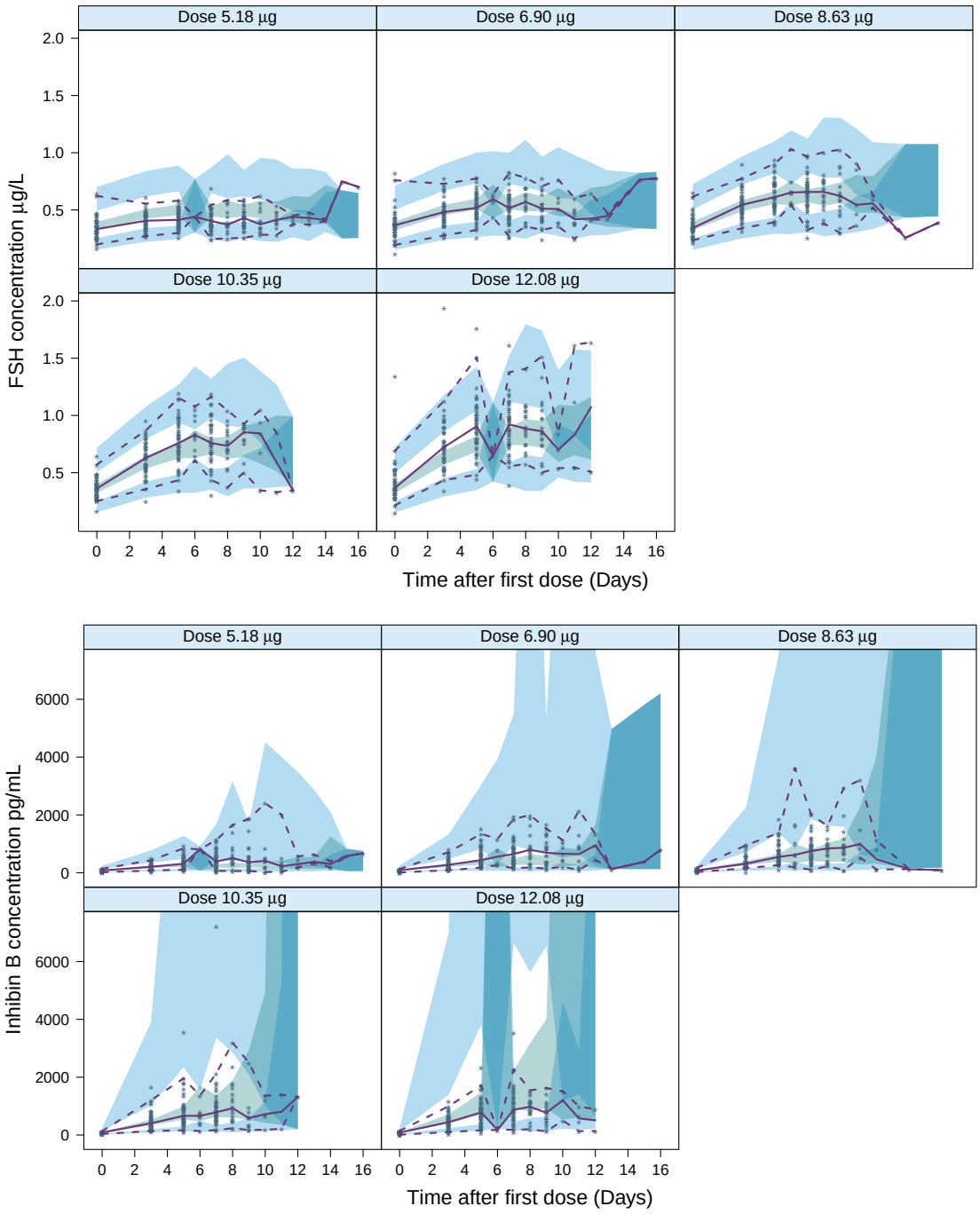

Figure 9.15: Visual predictive check for FSH at the top and inhibin B at the bottom from the PKPD model. The points are observations with purple lines for the 2.5th, 50th, and 97.5th percentiles of observations. The shaded areas are the simulated 95% confidence intervals (CI) for the 2.5th, 50th, and 97.5th percentiles.

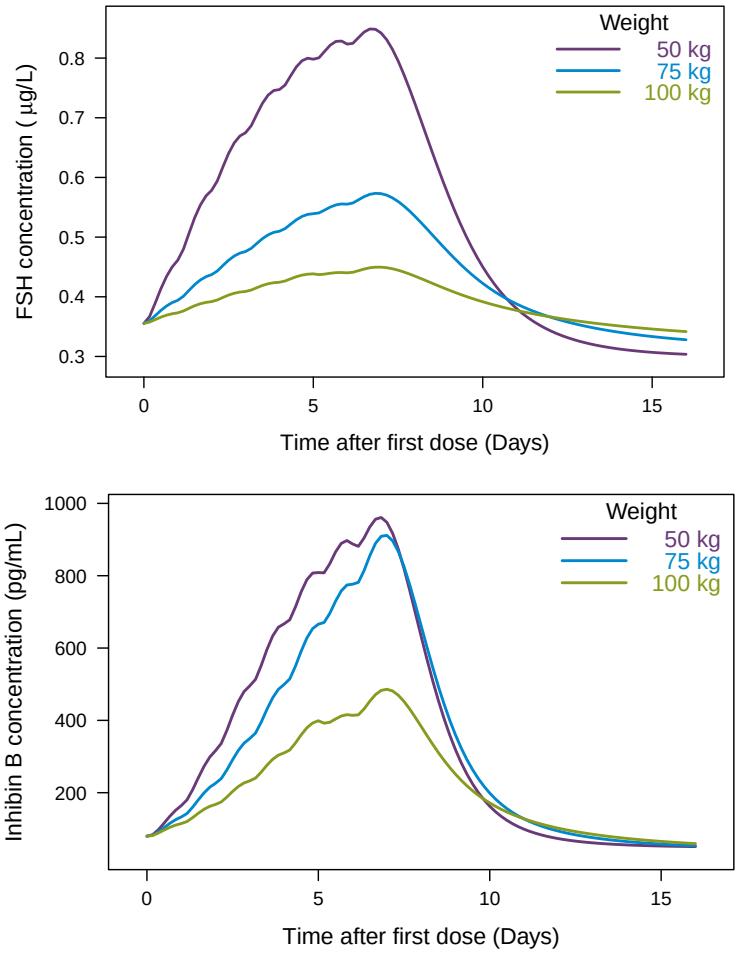

Figure 9.16: Illustration of simulated FSH (top) and inhibin B (bottom) concentrations for three patients of different weights. The patients received 7 doses of 10 μg FE 999049.

model. The resulting concentration-time profiles decrease with increasing body weight for both hormones (Figure 9.16).

A sigmoidal E_{max} dose-response model based on the 000009 study data was used for simulations to obtain 95% CI for the inhibin B response to the different doses. Compared to 95% CI of observations the interval is narrower with increased precision in the prediction (Figure 9.17). Due to the over- and under-predictions additional refinements may be needed before the simultaneous PKPD model is used for simulation of dose-response relationship.

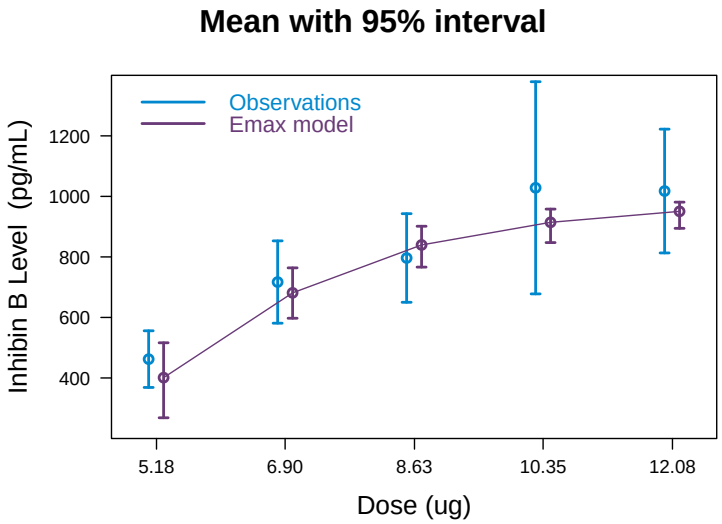

Figure 9.17: Illustration of how modelling of dose-response can increase precision in inhibin B predictions. Blue bars are 95 % confidence intervals (CI) of observations around the mean. The purple line is the mean model prediction with 95 % CI for the doses from simulations with a sigmoidal E_{max} dose-response model.

Chapter 10

Discussion & Perspectives

Drug development is an ongoing search for better, more efficient, cheaper, safer, or different drugs from existing ones, as well as developing new treatment strategies, or finding compounds for new therapy areas. For new compounds the minimal effective dose should be identified to avoid side effects. A drug with great efficacy might not be utilised to its full capacity if not administered optimally. What the optimal treatment strategy is, is very likely to differ between patients. Major challenges for individualising treatment are to identify how the dose should be adjusted based on patient-specific factors, what the dosing intervals should be, and whether changes should be made in long term treatment. An important tool for a more efficient and informative drug development path is using MBDD with a population modelling approach for analysing data, simulating different scenarios, and product comparison in order to assess the potential of a new drug and investigate likely treatment strategies. In addition, significance of potential factors influencing drug exposure and effect can be quantified. Thus MBDD can support decision making, dose selection, and study designs.

In this PhD thesis the PK and PD properties of FE 999049 were characterised through development of population PK and PKPD models based on clinical data. FE 999049 differ from marketed rFSH products by being expressed in a human cell line instead of a CHO cell line. The cause of infertility varies greatly, it is therefore important not only to find a safe dose but also an optimal individual dosing scheme according to patient-specific factors to increase success rate in pregnancy. Hence, there is a demand for infertility therapy drugs with innovative personalised treatment strategies to catch the great diversity in causes and extent of infertility.

Based on the first-in-human data with FE 999049 (CS01) an initial PK model was developed in paper I to investigate the pharmacokinetics of FE 999049 after single dose administration. The resulting model was a one-compartment distribution model with first order elimination and a delayed absorption through a transit compartment. The pharmacokinetics of a drug is characterised by its PK parameters, i.e. it should usually be described by one PK model. However, what can be observed and tested in the model and how many parameters that can be estimated is data driven, thus it depends greatly on the study design. Due to differences in baseline values, number of BQL measurements, and method for handling the BQL measurements, the first model from paper I could not directly be confirmed in paper II with the CS02 dataset. After adjusted according to differences in study

design, the overall model structure was confirmed in paper II. The PK datasets were relatively sparse and no i.v. data was used. That these data only support a one-compartment PK model for describing the pharmacokinetics of an FSH product is in agreement with previous results from the existing FSH models discussed in Chapter 6 section 6.1.1.

Using the knowledge about the reproductive hormone dynamics presented in Chapter 3 it was investigated if any of the ovarian hormones could be identified as a covariate in the model. It was possible to include the inhibitory effect of progesterone and inhibin B at endogenous FSH in the model developed from the CS02 data in paper II. As discussed in Chapter 4 section 4.2.1 inhibin B has been listed in the literature as one of the valuable predictors of ovarian response to gonadotropin therapy, and was therefore chosen as an interesting biomarker for assessment in this PhD. The observed influence of inhibin B at endogenous FSH in paper II was further investigated in paper III, where the dynamics between FSH and inhibin B were described by a simultaneous dose-concentration-response PKPD model based on the phase II 000009 data.

In all three models body weight was found to be a statistically significant covariate at CL/F and V/F, resulting in lower drug exposure with higher body weight. These findings are in accordance with the existing FSH modelling results presented in Chapter 6 and support the evidence for body weight being a factor affecting the pharmacokinetics of FSH compounds. In addition in paper III body weight was found to affect the absorption rate and the FSH stimulation of inhibin B.

The two lowest doses in the CS01 data did not seem to fulfil dose linearity as their mean profiles fell together (Figure 8.1) and 70% of the 220 measurements were BQL. It was considered if these doses at all would add any information to the analysis, or even worse cause bias of parameters. This could be true if the BQL measurements were ignored. The possibility of including BQL measurements is one of the advantages of population modelling. By using the M3 method the BQL measurements were accounted for, such that the information - even though limited - from the two lowest doses were utilised. No information is lost and less bias is induced as no inaccurate assumptions of the values are used, e.g. assuming they are zero or LLOQ/2 (see Chapter 5 section 5.4.1).

All pre-dose FSH measurements in the CS01 data were BQL indicating that endogenous FSH was successfully suppressed by the COCs. Nonetheless, three subjects showed an additional peak of FSH levels several days after FE 999049 administration, which was very unlikely to be caused by FE 999049.

There is no confirmatory conclusion for the reason of this second peak, a possible explanation is lack of adherence in these three subjects to oral contraceptives for suppression of endogenous FSH. As a first rule, all data should be included

but these three individuals were excluded from the analysis as their FSH levels were judged not to reflect the pharmacokinetics of the exogenously administered FE 999049.

The fact that choices of error and covariate model affect the structural model (see Chapter 5 section 5.5) was experienced in the model development in paper II. At first the model from paper I could not be used with the CS02 data. Adding a transit compartment to an initial basic model improved the parameter estimates but the OFV was worse. When the best covariate relationships were found and the endogenous FSH was properly described, the structural model was re-evaluated, and it was then significantly better with a transit compartment in the absorption process.

Differences in the absorption rates and their IIV were observed. In paper I an IIV was placed on k_a and in paper II the IIV was higher and at k_{tr}. Moving the random effect in any of the models to the other absorption rate constant resulted in a slightly higher OFV and worse precision. It was neither possible to have an IIV at both rate constants. There were indications of variation in both absorption rate constants but in the current studies it was not possible to identify both. When inhibin B was not included as a time-varying covariate in the model in paper II, the variation at k_{tr} increased. This could imply that if the endogenous FSH input is not described properly, the variation in FE 999049 absorption increases in order to explain the variation otherwise caused by differences in endogenous FSH levels between subjects.

Pooling of the two phase I data-sets and including i.v. data could maybe facilitate enough information for a two-compartment model to be supported by the data and a clearer identification of variations in the absorption processes.

Few samples per subject in the phase II 000009 data also affected the model development. Approximations, assumptions, and fixation of parameters were necessary in order to get a stable PKPD model in paper III. The PK information in the 000009 data was very sparse and not enough to estimate all PK parameters, so k_a and V/F were fixed to values from paper II. The PK parameters from paper II were chosen over the values from paper I, since the CS02 data include more FSH measurements and few BQL observations. In addition IC_{50} and k_{out} had to be fixed to stabilise the model.

The inclusion of a full covariance matrix indicates correlation between PK and PD parameters and significant variation between subjects. It was tested if IIV should be placed on more or other parameters, but no better combination was found. In the reproductive system FSH and inhibin B are correlated, thus it makes sense the model parameters describing the relationship are as well. In addition, other ovarian hormones are involved in the dynamics as described in Chapter 3. The over- and under-predictions observed can thus be due to that the

full endocrine dynamics is not considered. Furthermore, the data was not sufficient to support more advanced functions for describing the relations. In earlier models where an effect compartment was included and the FSH stimulation of inhibin B was modelled with an E_{max} model, the VPCs were better with less over- and under-prediction. In spite of the better fit such models were discarded due to poorly estimated parameters and model instability. More data would probably be needed to improve the model and for describing the FSH-inhibin B relationship more accurately, which might require more complicated functions, e.g. an E_{max} model for the FSH stimulation of inhibin B production rate.

Subjects did not stay in the trial the same number of days, which is not accounted for in the model and can add to the reason for imprecise predictions at high values. An option could be to model this as drop-out subjects. A few extremely high inhibin B concentrations were observed, so it was looked into if these affected the results. The subjects with extreme values were identified and excluded but it did not improve the model or change the VPCs.

Through simulations the models were used to predict outcomes of different doses to women of different body weights. In all three models the simulations were performed for three subjects weighing 50 kg, 75 kg, and 100 kg who received 7 daily doses of FE 999049. Consistently the simulated FSH exposure decreased with increasing body weight (Figure 9.5, 9.12, 9.16). The second PK model based on CS02 data included an endogenous FSH supply and initial exposure levels therefore started above zero and was likewise slightly higher over time compared to the first PK model based on CS01 data where no endogenous FSH levels were measurable. The PKPD model was based on patient data from a phase II trial. The patients would likely have different endocrine profiles and they have initial higher endogenous levels than the gonadotropin suppressed female volunteers in the phase I trials. This is also reflected in the simulations where the PK profiles look different from the ones obtained with the PK models. After dosing the FSH exposure decrease to an endogenous level. In the PKPD model it can become lower than pre-dose levels due to suppression by the GnRH antagonist and inhibin B levels. Since FSH stimulates the inhibin B production the simulated inhibin B response in the PKPD model also decrease with increasing body weight. In addition, body weight affects the FSH stimulation of inhibin B thus the extent of difference between the concentration-time profiles are different for inhibin B response compared to FSH exposure. Body weight play a role in the variation in exposure and thus response but other factors also affect the exposure. Endogenous FSH and variation in its level over time is likely an influential factor too.

The PKPD model would need more work before further simulations can be performed. Then, the potential of inhibin B as a marker for ovarian response could be investigated and be related to later clinical PD endpoints like oocytes

retrieved. It could be interesting to test the hypothesis from the literature discussed in Chapter 4 about how poor and good responders are related to inhibin B levels and changes during treatment. There are evidence of inhibin B being the first PD marker measurable during infertility treatment with FSH products and an indicator for initial follicle development in response to FSH. Hence, it could be useful to be able to predict inhibin B levels in a patient to determine if the dose is effective. Individual FSH thresholds cannot be measured easily but when simulating both FSH and inhibin B, the inhibin B increase implies that follicles have started their gonadotropin dependent growth and thus the FSH threshold has been surpassed. In addition, the model could be used to simulate the typical dose-response relationship for inhibin B to see what dose range is required to get a proper response in inhibin B. If the inhibin B response is not sufficient it indicates that no antral follicles have been recruited and entered the gonadotropin dependent growth phase.

In decision making and dose selection is needed to know the response of different doses. A pre-set criterion for a minimum response or a desired response interval can be set. If only looking at observed data it can be difficult to determine whether the criterion has been met due to an often large variation in the population. Modelling and simulation can potentially improve precision by predicting typical response. Using a sigmoidal E_{max} dose-response model based on the 000009 study data, better precision in the predicted response to different doses was achieved compared to confidence intervals for observed data obtained with traditional statistical methods. The precision would be expected to improve further when using the semi-mechanistic PKPD model to simulate the FE 999049 dose-inhibin B response compared to when using the empirical sigmoidal E_{max} dose-response model, and can thus improve decision making and provide a better tool for dose selection.

Chapter 11

Conclusions

Data from three clinical trials were used to characterise the PK and PD properties of FE 999049, a novel human rFSH for controlled ovarian stimulation in ART. Using a population approach with nonlinear mixed effects models the PK profile of FE 999049 was successfully described by a one-compartment model, both after single and repeated administration. A semi-mechanistic PKPD model was developed to describe the dynamics between rFSH, inhibin B, and endogenous FSH. Inhibin B was chosen as the PD endpoint because it has been suggested to be the earliest response marker to FSH treatment and an indicator of follicular growth.

From these model-based exploratory analyses it can be concluded that

1. Body weight
   - is a statistically significant covariate explaining some of the variation in the PK parameters CL/F and V/F in the models.
     - The resulting effect is that FE 999049 exposure decrease with increasing body weight: A patient weighing twice as much as another patient would need a 1.8 times higher dose to get the same FSH concentration.
   - affects the FSH stimulation of inhibin B in the PKPD model.
     - With higher body weight the same FSH concentration has a higher stimulatory impact on inhibin B production rate.
2. Endogenous FSH levels
   - can significantly contribute to the measured serum FSH concentrations, hence if not accounted for in the model
     - the FSH exposure is under-predicted.
     - a bias is induced in the PK parameter estimates.
   - depends on inhibin B and progesterone levels.
   - change over time and has to be described properly in the model, otherwise
     - unexplained parameter variation increase, for the absorption rate constant it is doubled.

  - a transit compartment cannot be included, thus the structural model change.
  - a bias is induced in the PK parameter estimates.
- are thus not constant throughout treatment and baseline correcting data might be wrong.

3. Inhibin B

- significantly inhibit endogenous FSH production rate.
- production rate constant is stimulated by total FSH levels.
- response decrease with increasing body weight.
- levels vary greatly between subjects.

These results support the current findings presented in the literature of body weight being an important factor in dosing of FSH products and resulting exposure. Furthermore, it was identified that inhibin B response, stimulated by FSH exposure, also decrease with increasing body weight. The advantage of the population PK models developed in this PhD work is that they can be used for simulating different scenarios of body weight and dose, they take into account the endogenous FSH for more accurate estimation of parameters and prediction, and have specifically identified variation in the population parameters.

The PKPD model has the potential of predicting a specific patients PK and PD profile, simulate overall dose-response relationship, and hereby aid in decision making. For FSH products in the development phase, such a model can give indications of required dosing range or support a go/no-go decision based on if a sufficient inhibin B response can be achieved.

Thus, population PKPD modelling is a useful tool for analysing clinical data and the possible applications seems endless. Even though MBDD is a growing field it is not utilized fully in the pharmaceutical industry. Overall in this thesis it was demonstrated how population modelling can be used to gain information from clinical data and it has been emphasised that many disciplines need to be integrated in order to produce reliable and useful models.

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Paper I

Population Pharmacokinetic Modelling of FE 999049, a Recombinant Human Follicle Stimulating Hormone, in Healthy Women after Single Ascending Doses

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Abstract

Background and Objective

The purpose of this analysis was to develop a population pharmacokinetic model for a novel recombinant human follicle stimulating hormone (FE 999049) expressed from a human cell line of fetal retinal origin (PER.C6®) developed for controlled ovarian stimulation prior to assisted reproductive technologies.

Methods

Serum FSH levels were measured following a single subcutaneous FE 999049 injection of 37.5, 75, 150, 225, or 450 IU in 27 pituitary suppressed healthy female subjects participating in this first-in-human single ascending dose trial. Data was analysed by nonlinear mixed effects population pharmacokinetic modelling in NONMEM 7.2.0.

Results

A one-compartment model with first order absorption and elimination rates was found to best describe the data. A transit model was introduced to describe a delay in the absorption process. The apparent clearance and volume of distribution estimates were found to increase with body weight. Body weight was included as an allometrically scaled covariate with a power exponent of 0.75 for clearance and 1 for the volume of distribution.

Conclusions

The single dose pharmacokinetics of FE 999049 were adequately described by a population pharmacokinetic model. The average drug concentration at steady-state is expected to be reduced with increasing body weight.

Key Points

- The population pharmacokinetics of the novel recombinant human follicle stimulating hormone FE 999049 have been characterised in healthy females after single ascending dosing
- Follicle stimulating hormone measurements below the quantification limit were accounted for by the model.
- Body weight influences exposure to FE 999049 and may be an important factor for dosage considerations.

1. Introduction

The female reproductive function is controlled by periodically regulated production, secretion, and interaction of hormones in the hypothalamic-pituitary-gonadal axis. Of particular importance are the two gonadotropins follicle stimulating hormone (FSH) and luteinizing hormone (LH), both of which are released from the anterior pituitary gland upon stimulation by gonadotropin-releasing hormone (GnRH) from the hypothalamus. During the normal menstrual cycle, the combined actions of FSH and LH induce development of a single dominant follicle, ovarian hormone production, oocyte maturation, and ovulation.

In the management of infertility, exogenous FSH administration is used to induce monofollicular development and ovulation in anovulatory women [1], or multiple follicular development to allow selection of embryos for transfer in women undergoing in vitro fertilisation (IVF)/intracytoplasmic sperm injection (ICSI) treatment [2].

Urinary menopausal gonadotropin preparations that contain both FSH and LH activity are being extensively used for controlled ovarian stimulation (COS) in IVF/ICSI treatment. In 1989 advances in DNA technologies enabled development of a recombinant FSH (rFSH) generated in Chinese Hamster Ovarian (CHO) cell lines which expressed the genes encoding human FSH [3]. Since then several rFSH products have been marketed including two original rFSH compounds originating from CHO cell lines [4;5] and a long acting rFSH [6].

A novel recombinant human FSH (rhFSH, FE 999049) expressed from a human fetal retinal cell line (PER.C6®, Crucell, Leiden, The Netherlands) is under development by Ferring Pharmaceuticals A/S for patients undergoing COS for IVF/ICSI. Dose proportionality has been shown for maximum concentration (C_{max}) and area under the concentration-time curve (AUC) by non-compartmental analysis (NCA) of data from both a single ascending dose phase 1 trial with Caucasian women and one with Japanese women [7]. The purpose of this analysis was to describe the population pharmacokinetics of FE 999049 based on the Caucasian trial in Olsson et al.[7]. When using a population modelling approach with nonlinear mixed effects models, as opposed to NCA, it is possible to investigate variation in the population and to identify potential covariates explaining some of the variability in drug exposure.

2. Materials and Methods

2.1 Clinical trial design

The first-in-human trial with FE 999049 was a randomised, double-blind, placebo controlled, sequential single dose escalation trial investigating the safety, tolerability, and pharmacokinetics. The trial was carried out in accordance with the Declaration of Helsinki and Good Clinical Practice. It was approved by regulatory authorities and local ethics committees. All subjects gave written informed consent to participate. The trial is the 'Caucasian study' described in Olsson et al. [7]. In summary, the trial population consisted of 40 healthy female volunteers aged 21-35 years with a normal menstrual cycle and a body mass index (BMI) ranging 18-29 kg/m^2 (Table 1). They received a single subcutaneous abdominal injection of 37.5, 75, 150, 225, or 450 IU FE 999049 or placebo. In each dose group there were 8 women whereof 2 were given placebo.

To avoid any interference with endogenous FSH levels during the trial, all volunteers were pituitary down-regulated by means of a combined oral contraceptives (COC). To ensure similar down-regulation in all subjects, they were all switched from their COC to the same high-dose COC (OGESTREL 0.5/50, 50 µg of ethinyl estradiol, 0.5 mg norgestrel, Watson Pharma Inc.) 14 days before drug administration.

Blood samples for measurement of serum FSH concentration were collected 60 and 30 minutes prior to administration, immediately before administration, at every 4 hours the first 48 hours and subsequent every day up to 9 days after administration. Determination of serum FSH concentrations was performed at Ferring Pharmaceuticals A/S with a validated immunoassay based on electrochemiluminescence (MSD sectorTM Imager 2400) with a lower limit of quantification (LLOQ) of 0.075 µg/L.

2.2 Data

FE 999049 was dosed in IU and for this analysis converted to µg units (2.2, 4.4, 8.8, 13.1 and 26.3 µg) using the specific activity in order to estimate the serum concentrations of FE 999049 in µg/L units.

Prior to the modelling, mean serum FSH concentrations versus time were plotted for each of the 5 treatment groups including all subjects (Figure 1). A second increase in FSH concentration starting at day 3 after administration was observed for the 4.4 µg dose group. From the individual concentration-time profiles (not shown) two subjects were identified to cause this increase. One subject in the 8.8 µg dose group had a second increase in FSH concentration at day two. Their

concentration-time-profiles were considered not to reflect the pharmacokinetic (PK) profile for the exogenously administered rhFSH (FE 999049) and were excluded from the analysis. A total of 594 samples from 27 individuals were included in the analysis. 258 measurements, constituting over 40% of the total data points, were below the quantification limit (BQL). In the 2.2, 4.4, 8.8, 13.1 and 26.3 µg dose group 67, 74, 39, 23, and 23% of the measurements were BQL, respectively.

2.3 Pharmacokinetic Modelling

A population PK model was developed using nonlinear mixed effects modelling. This included finding a structural model together with appropriate error models describing interindividual and residual variability. To increase the predictive capability of the model, it was checked if part of the interindividual variability (IIV) in parameter estimates could be explained by covariates (body weight, age, and dose).

Model development was guided by changes in the NONMEM objective function value (OFV), precision of parameter estimates, and graphical model goodness-of-fit assessments including visual predictive checks (VPC). The OFV is approximately proportional to -2log likelihood. The difference in OFV between two nested models is approximately χ^2-distributed, with degrees of freedom equal to the difference in the number of parameters. Based on this, the statistical significance for inclusion/exclusion of a model parameter can be judged. For this descriptive analysis a significance level of 0.05 was used for discrimination among nested models and covariate testing.

BQL measurements were accounted for in the analysis using the M3 method since for a high proportion of BQL it is the preferred method out of the 7 existing methods [8-10]. With the M3 method BQL data are censored observations and treated as categorical data. These are included in the likelihood function for the model parameter estimation as the likelihood that the observation is truly BQL. The sensitivity of the model parameter estimates to the BQL method used was evaluated by comparing the estimates from the final model to those estimated when the BQL measurements were ignored (M1), set to LLOQ/2 (M5), or set to zero (M7).

The final model was used for simulations to illustrate the FE 999049 concentration-time profile and steady state exposure following repated administartion of 10 µg. The average steady state exposure was calculated as

$$C_{ss,av} = \frac{10\ \mu g}{CL/F * 24h}$$

2.4 Software

The models were implemented and parameters estimated in NONMEM 7.2.0 (Icon Development Solutions, USA) [11] using first order conditional estimation with interaction. Data handling and graphical representations were performed in R version 2.11.1 [12]. VPCs were performed using PsN [13;14] and plotted using Xpose [15].

3. Results

A one-compartment distribution model with first order absorption and a transit model for adding a delay in the absorption was found to adequately describe data. The time-course of serum FE 999049 concentration after dosing was described by the differential equations (1) - (3), one for each of the three compartments representing the dosing site, the transit model, and the central compartment, respectively.

$$\frac{dA_1(t)}{dt} = -k_{\mathrm{tr}}A_1(t) \quad (1)$$

$$\frac{dA_2(t)}{dt} = k_{\mathrm{tr}}A_1(t) - k_aA_2(t) \quad (2)$$

$$\frac{dA_3(t)}{dt} = k_aA_2(t) - kA_3(t) \quad (3)$$

At time t, $A_i(t)$ is the FE 999049 amount in the i^{th} compartment. The absorption rate from the dosing site and the transit compartment is k_{tr} and k_a, respectively. The elimination rate of FE 999049, k, is clearance (CL) divided by the volume of distribution (V). Since it is the amount tracked in the equations, predicted serum FE 999049 concentrations are calculated as $A_3(t)$/V. Data is obtained after subcutaneous dosing so the bioavailability (F) is not known. The CL and V estimated here are therefore the apparent clearance (CL/F) and apparent volume of distribution (V/F). A combined additive and proportional error model was used to describe the residual error.

The parameters CL/F, V/F and k_a varied between subjects. A variation in k_{tr} was also observed, but given the current data it was not possible to include separate IIV on both parameters describing the absorption process. It was chosen to keep the variability on k_a because this model had the best OFV

compared to the model with variability on only k_{tr}. For parameters with IIV the i^{th} subject's individual parameter, P_i, is

$$P_i = P_{tv} \exp(\eta_i) \quad , \tag{4}$$

where P_{tv} is the typical population parameter and η_i is the individual random effect from an approximately normal distribution with mean zero and variance ω_p^2 for the IIV. A positive correlation was identified between CL/F and V/F by a statistically significant improvement in OFV when adding a covariance between the two parameters.

Body weight was found to be a statistically significant covariate on CL/F and V/F and was included in the model parameters as

$$P_i = P_{tv} \exp(\eta_i) \left(\frac{\mathrm{WT}_i}{65\ \mathrm{kg}}\right)^{AL_P} \tag{5}$$

where WT_i is the i^{th} subject's body weight and AL_p is the allometric values: 0.75 for CL/F and 1 for V/F. Adding body weight as a covariate caused a drop of -2.94 in OFV which was considered significantly better since no extra parameters were added. In addition, the coefficient of variation (CV) for the unexplained IIV was reduced from 31.4 to 28.2% CV for CL/F and from 46.4 to 44.3% CV for V/F. Not including body weight in the final model at either CL/F or V/F increased the OFV by 10.8 and 4.4, respectively. Subject age did not further explain any of the IIV. There was neither any influence of dose at the parameters indicating that the pharmacokinetics is linear.

The final model parameters are listed in Table 2. The mean observed FSH data and typical model predictions are shown for each dose level in Figure 2a. In the diagnostic VPC plot (Figure 2b) the observed data is compared to model predictions based on 1000 simulated trial datasets using the final model. Since the data consisted of more than 40% BQL measurements that were included in the model by the M3 method, a two panel VPC illustrating both continuous and categorical (BQL) data was used[10]. The top panel displays the observations above LLOQ. The 2.5th, 50th, and 97.5th percentiles of observations and the 95% confidence intervals (CI) for the corresponding model predictions are plotted. In the bottom panel the observed and predicted proportion of BQL observations are visualised. Both the points for observations against individual and population predictions fall around the unity line and there is no trend observed in the model residuals (Figure 3).

When re-estimating the final model parameters using a simpler method than M3 for handling BQL measurements slight changes in the parameter estimates were observed. With the M1 or M5 method the PK parameters changed by less than 10% from the parameters obtained with the M3 method. On the contrary, applying the M7 method instead of M3 caused greater parameter changes from the values in the final model and the RSEs increased. The largest change was an increase in the CL/F estimate by 22% to 0.524 L and with a fourfold increase in its RSE. Thus making M7 the least precise method for this model. The impact of body weight on the expected FE 999049 concentration following multiple dose administration was investigated using the final model for simulations. In Figure 4a, illustrating the time-course of FE 999049 concentration in three subjects of different weights, it is shown how the concentration decreases with increasing body weight. Taking IIV into considerations there are large overlaps in the average steady state concentrations across the three weight groups (Figure 4b). However, the FSH exposure appears to be lower in the majority of subjects with body weight of 100 kg compared with FSH levels in subjects with body weight of 50 kg.

4. Discussion

In the present study, population PK modelling was carried out to characterise the pharmacokinetics of FE 999049 after single ascending doses in healthy women. The objective was to get an initial understanding of the time-course of drug exposure and the magnitude of inter-individual variability through a modelling approach. In addition, to optimise ovarian response to treatment with FE 999049, it was examined whether patient-specific variables can aid in the design of individualised dosing schemes.

The pharmacokinetics of FE 999049 were described by a one-compartment distribution model with first-order absorption and elimination. These findings are in accordance with previous results that have shown that FSH, either as urinary or recombinant preparation, follows a one-compartment model after s.c. or i.m. administration [16-20]. Some of these studies have found exogenous FSH pharmacokinetics to be best described by a two-compartment model if the data is “rich”, i.e. with extensive number of samples, or if doses are given intravenously [17-19]. A two-compartment distribution model was also tested here. As this was a first single dose trial with few subjects, the data generated was not sufficient to give successful estimation of the extra parameters for the peripheral distribution compartment.

A transit model with one compartment was introduced to describe a somewhat prolonged absorption of FE 999049 causing an apparent delay for the measurable change in serum FE 999049

concentration. It is conceivable that this extra transit time in the absorption process could be attributed to the lymphatic system, since proteins given subcutaneously are usually absorbed through the lymphatic system [21]. It was tested whether the FE 999049 absorption could be described by alternative models. Adding an extra transit compartment in the final model increased the OFV by 0.002 and is thus worse. Using a lag-time instead of a transit compartment decreased the OFV by 0.085, but these two models are not nested and the OFV cannot be compared with statistically significance. Since the models have the same number of parameters and basically no difference in neither OFV nor the model fit graphs, it was chosen to keep the transit model because it is more mechanistic correct than a lag-time.

As part of the model development it was checked whether any covariates could be identified. Body weight was found to be the cause of some of the variation in FE 999049 concentration after treatment. It is consistent with previous analyses that have shown a relation between serum FSH and the PK parameters with body weight [16;17;22;23]. In the current study, with the power exponent fixed to allometric values, body weight was a significant covariate and could explain some of the IIV in CL/F and V/F indicated by a reduction from 31.4% CV and 46.4% CV to 28.2% CV and 44.3% CV, respectively. The marginal effect of adding body weight as a covariate is likely due to the limited number of subjects in this first-in-human trial with a relatively narrow body weight range. Among the potential covariates (age, body weight, and dose) body weight was the only covariate identified.

In order to avoid interference with endogenous FSH in the analysis of the FE 999049 pharmacokinetics, all subjects in this trial were pituitary suppressed by means of COC. Since all pre-dose FSH measurements were BQL the measured serum FSH concentrations were exclusively reflecting exogenous FSH from FE 999049. Nonetheless, three subjects showed an additional peak of FSH levels several days after administration of FE 999049, which could likely be due to endogenous FSH levels not being fully suppressed. These individuals were excluded from the analysis as their FSH levels were judged not to reflect the pharmacokinetics of the exogenously administered FE 999049. The initiation of the additional FSH peak occurred 3 days after FE 999049 administration which was the day after the trial subjects were discharged from the residential stay in the clinic. A possibly explanation for the later secondary increase in FSH levels could be poor compliance to taking OGESTREL after discharge from the clinic, however, such protocol deviation was not reported.

In future studies where subjects are not pituitary suppressed or down-regulated, endogenous FSH levels have to be considered in the modelling. Especially in phase 2 studies where patients could have varying and measurable endogenous FSH levels influencing the total FSH concentration. When

available, the significance of other reproductive hormones such as inhibin B, estradiol, and progesterone should also be studied. Since these hormones influence FSH levels over time they could potentially further explain the observed variation in the FSH concentration profile between subjects.

The identified lower drug exposure with higher body weight should be further quantified in future models from other clinical studies with FE 999049. It must also be related to any subsequent effects that possibly could add variability in clinical efficacy endpoints (e.g. number of oocytes retrieved, successful implantation rate and pregnancy rate) in order to judge if there is a therapeutic value in individualising dosing based on patient's body weight. Modelling has previously been used to set two different rFSH dosage regimens for subjects weighing more or less than 60 kg [16;24].

5. Conclusion

A population PK model was successfully developed for FE 999049 using data from a single ascending dosing trial in healthy female subjects. There were indications of that FE 999049 exposure decreases with increasing body weight. When also considering findings from the literature body weight can be an important factor to consider in efforts to develop individualised dosing regimens for optimised treatment outcomes. However, in order to confirm the influence of body weight at FE 999049 exposure the model should be updated using data from subsequent clinical trials including multiple dose trials and trials involving patients, where the body weight range is likely to be wider. In addition the relationship between drug exposure and clinical efficacy/safety parameters must be established.

Compliance with Ethical Standards

Funding: This work was supported by Innovation Fund Denmark (Industrial PhD case number 11-117436).

Conflicts of Interest: Trine Høyer Rose, Daniel Röshammar, Lars Erichsen, and Lars Grundemar are all current or former employees at Ferring Pharmaceuticals A/S.

Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent: Informed consent was obtained from all individual participants included in the study.

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Table 1. Summary of subject characteristics

Dose (µg)	**2.2** n=6	**4.4** n=6	**8.8** n=6	**13.1** n=6	**26.3** n=6
Age (years)	30.2 (21-35)	29.5 (24-34)	26.2 (22-31)	30.2 (24-34)	27.3 (21-32)
Height (cm)	158.5 (154.9-166.0)	164.5 (157.5-175.3)	167.4 (154.9-182.9)	159.1 (152.4-162.6)	161.6 (152.4-170.2)
Weight (kg)	59.4 (51.6-75.4)	70.8 (65.9-80.3)	68.4 (51.8-90.0)	61.5 (54.5-69.1)	61.6 (52.5-68.6)
BMI (kg/m^2)	23.5 (21.5-27.4)	26.2 (22.9-28.6)	24.3 (19.7-28.9)	24.3 (21.3-27.9)	23.7 (18.7-27.2)

Personal demographics for all the subjects in the five dosing groups. The values are mean with range in brackets. n: number of subjects in each group, BMI: body mass index.

Table 2. Pharmacokinetic parameter estimates

Parameter	**Estimate**	**(RSE%)**	**IIV CV%**	**(RSE%)**	**Shrinkage (%)**
CL/F (L/h)	0.430	(6.3)	28.2	(29.5)	5.31
V/F (L)	28.0	(9.1)	44.3	(20.7)	4.82
k_{tr} (h^{-1})	0.517	(24.8)			
k_a (h^{-1})	0.160	(12.9)	23.3	(27.8)	31.8

Typical population pharmacokinetic parameter estimates obtained from modelling with the relative standard error (RSE) in brackets. F: bioavailability, CL/F: apparent clearance, V/F: apparent volume of distribution, k_{tr}: absorption rate from the dosing site to the transit compartment, and k_a: absorption rate to the central compartment. For CL/F and V/F the value is the typical value for a woman weighing 65 kg. The interindividual variability (IIV) is listed as the coefficient of variation (CV) with RSE in brackets and corresponding ETA shrinkage in percentage.

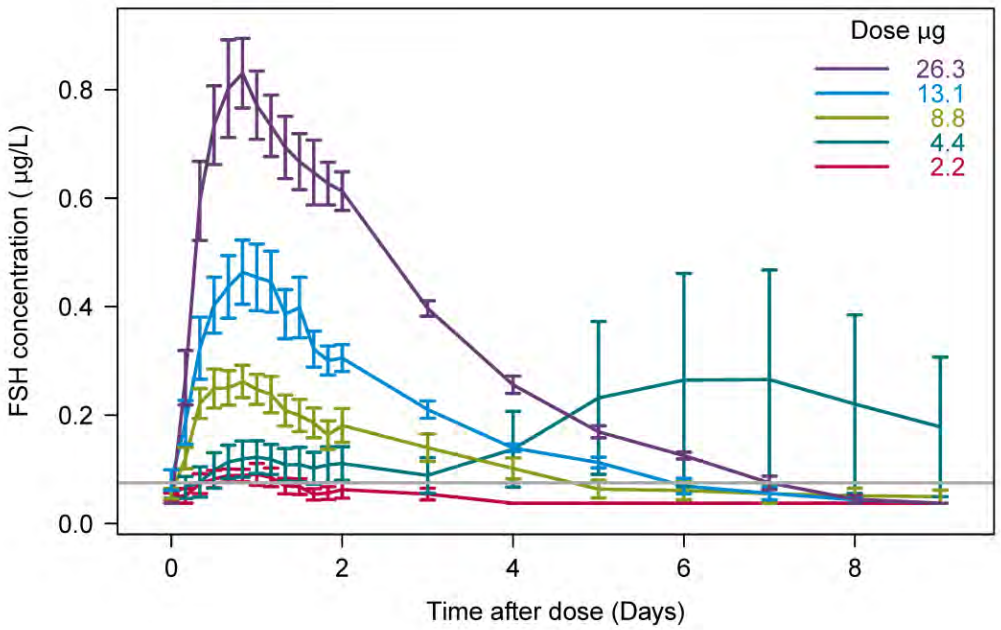


Figure 1. The observed FSH concentration shown as mean of all subjects with standard error (SE) bars for each treatment group. The grey line represents the LLOQ of 0.075 µg/L. Observed BQL measurements were plotted as LLOQ/2.

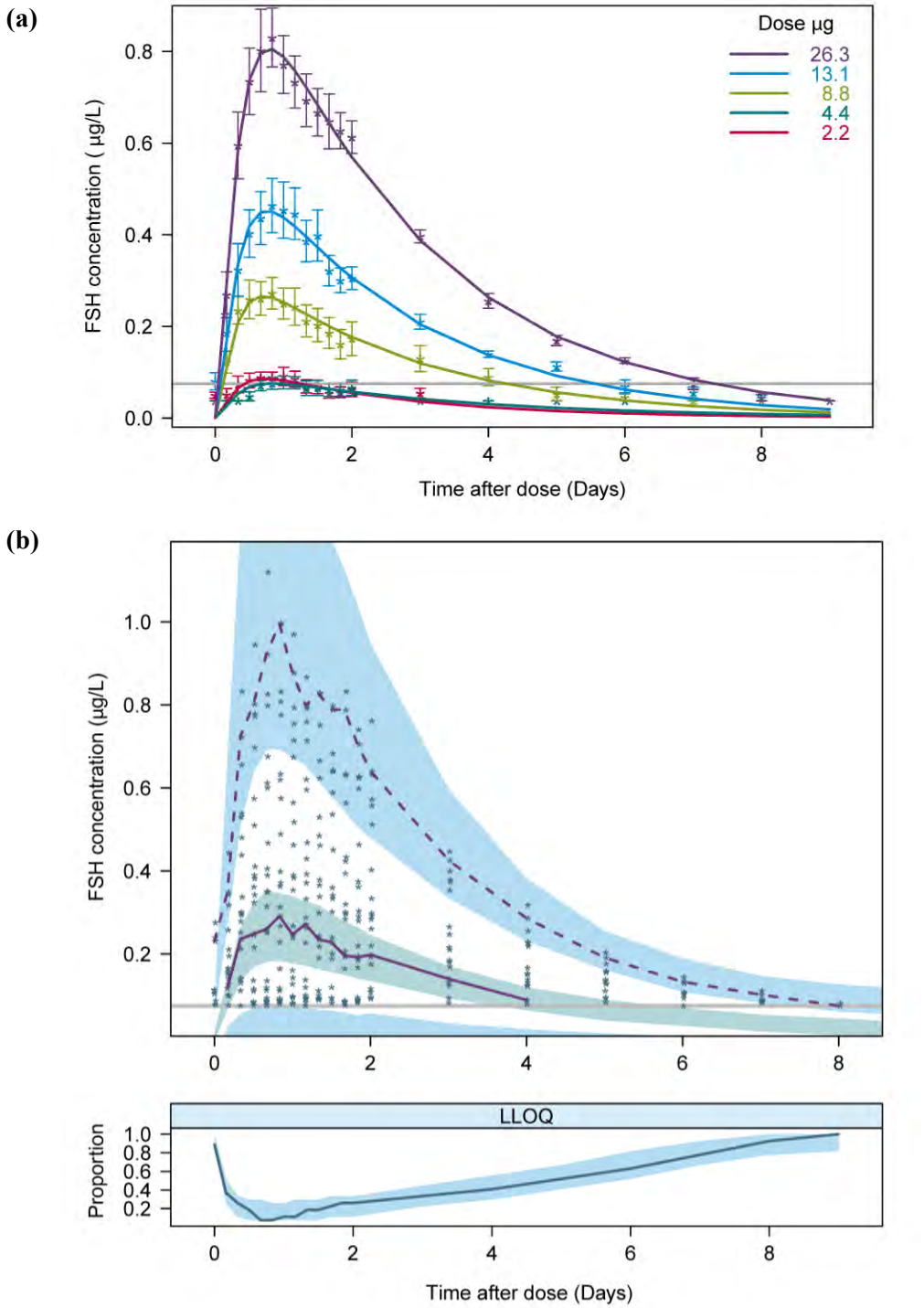
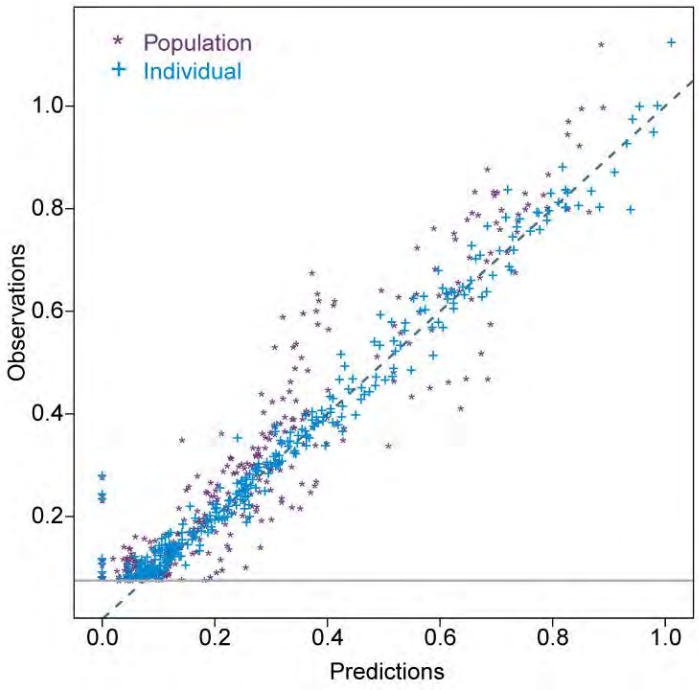


Figure 2. Model predictions compared to observations. **(a)** Points are mean of observations with standard error (SE) bars. Lines are typical model predictions for each treatment group. The grey line represents the LLOQ of 0.075 µg/L. Observed BQL measurements were plotted as LLOQ/2. **(b)** Two panel visual predictive check for all dose groups together. The top panel shows the observations above LLOQ (points) and the 50th and 97.5th percentiles of observations (purple lines). The 2.5th percentile of observations is not shown since it solely consists of BQL points. The shaded areas are the simulated 95% confidence intervals (CI) for the 2.5th, 50th, and 97.5th percentiles. The grey line represents the LLOQ of 0.075 µg/L. In the bottom panel the blue line is the fraction of BQL observations with the 95% CI for the median from simulations.

(a)



(b)

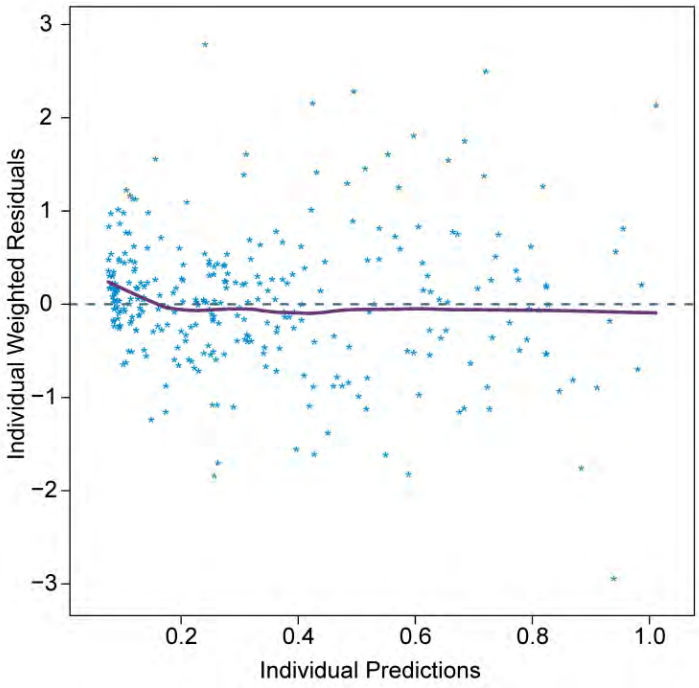
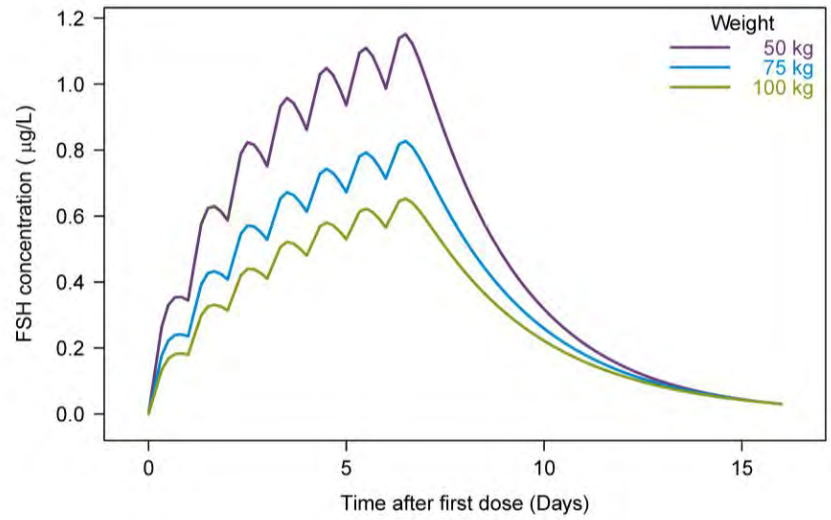


Figure 3. Goodness of fit plots. **(a)** Observations against population predictions (purple points *) and individual predictions (blue points +) with the unity line. The grey line represents the LLOQ of 0.075 µg/L. **(b)** Individual residuals against individual predictions (points) with a smooth lowess line.

(a)



(b)

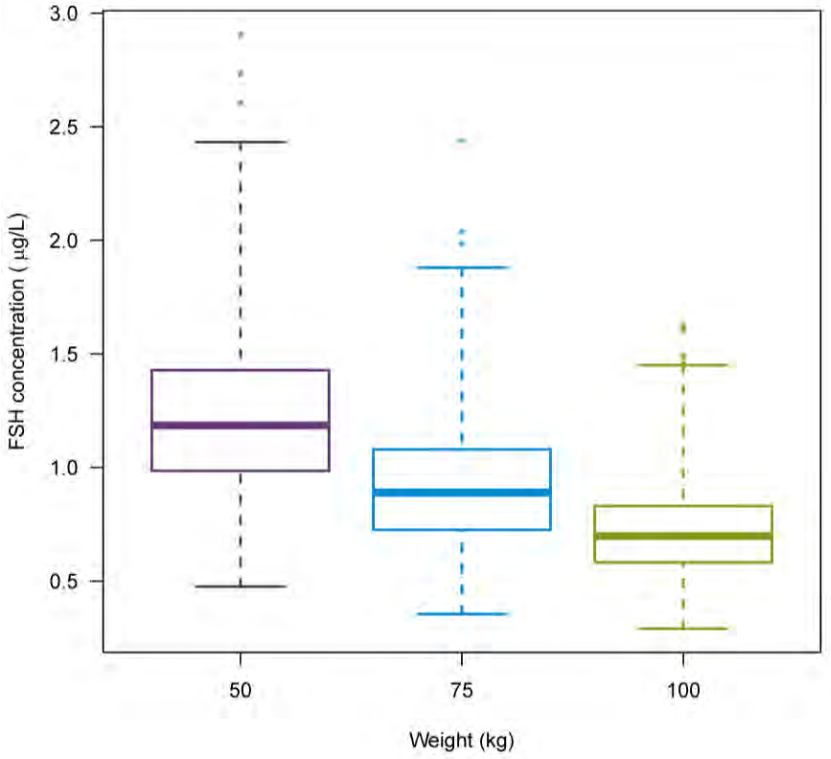


Figure 4. Effect of body weight at the FSH concentration. **(a)** Simulation of the typical expected FSH concentration after multiple dosing of 10 µg FE 999049 for three subjects with different body weights. **(b)** Boxplot of the average steady state concentration obtained from 1000 simulations for each weight group.

Paper II

Characterisation of Population Pharmacokinetics and Endogenous Follicle Stimulating Hormone (FSH) Levels after Multiple Dosing of a Recombinant Human FSH, FE 999049, in Healthy Women

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Abstract

Background and Objective

To characterise the population pharmacokinetics of FE 999049, a novel recombinant human follicle stimulating hormone (rhFSH), after multiple dosing in healthy women considering endogenous FSH levels.

Methods

Longitudinal measurements of FSH, luteinizing hormone, progesterone, estradiol, and inhibin B levels were collected after repeated subcutaneous dosing with 225 IU FE 999049 in 24 gonadotropin down-regulated healthy women participating in a phase 1 trial. The FSH population pharmacokinetics were evaluated using nonlinear mixed effects modelling and NONMEM 7.2.0.

Results

The measured FSH levels were modelled as a sum of endogenous FSH and the administered FE 999049. In this analysis the FE 999049 population pharmacokinetics were best described by a one-compartment model with first order absorption and elimination. A delay in the absorption was described by a transit model. The apparent clearance and volume of distribution was found to increase with body weight in accordance to an allometrically scaled power exponent of 0.75 and 1, respectively. The endogenous FSH levels were described by a turnover model. Endogenous FSH baseline levels were observed to be lower in individuals with higher baseline progesterone levels. The endogenous FSH levels were further suppressed over time with increasing inhibin B levels.

Conclusions

It can be of importance to account for endogenous FSH levels for accurate estimation of exogenously administered FSH pharmacokinetic parameters. Moreover, the endogenous FSH levels can be affected by reproductive hormones with time. Thus, correcting measured total FSH concentrations by the observed endogenous FSH baseline value at all time points may be incorrect.

Key Points

- The multiple dose pharmacokinetics of FE 999049 have been described accounting for endogenous follicle stimulating hormone (FSH) levels.
- The exposure to FE 999049 was influenced by body weight. Endogenous FSH levels were influenced by progesterone and inhibin B levels.
- When characterising the pharmacokinetics of recombinant FSH products the time varying contribution of endogenous FSH may be important to consider.

1. Introduction

Follicle stimulating hormone (FSH) is a gonadotropin synthesised and secreted by the anterior pituitary gland. The major function of FSH is to regulate the reproductive processes by stimulating the gonads. In females, FSH stimulates follicular development in the ovaries and production of inhibin B, progesterone, and oestrogens by the ovarian follicular granulosa cells. Luteinizing hormone (LH), another gonadotropin from the anterior pituitary, stimulates the theca cells of the follicles to deliver androgens to the granulosa cells for conversion to oestrogens. LH is also responsible for ovulation of the dominant follicle that has reached a full mature preovulatory stage. The ovarian hormones promote further follicular development as well as exerting negative and positive feedback loops to the hypothalamus and pituitary affecting the gonadotropin production and secretion. In addition, gonadotropin secretion is stimulated by gonadotropin-releasing hormone (GnRH) produced in the hypothalamus.

Female infertility can be caused by numerous factors at any level from the hypothalamus to the ovaries and uterus. Gonadotropin therapy with either menotropins or recombinant FSH (rFSH) preparations can be used for infertility treatment when the cause is not primary ovarian failure, such that the ovaries are still responsive with primordial follicles. The purpose of controlled ovarian stimulation with daily administration of gonadotropins prior to assisted reproductive technologies such as in vitro fertilisation (IVF) or intracytoplasmic sperm injection (ICSI) is to obtain an adequate number of oocytes per retrieval with the minimum risks for the woman [1]. An appropriate ovarian response leading to availability of several embryos makes it possible to select the best one(s) for transfer.

Recently, a novel recombinant human FSH (rhFSH, FE 999049, Ferring Pharmaceuticals A/S) has been expressed for the first time in a human cell line (PER.C6®, Crucell, Leiden, The Netherlands), while existing rFSH preparations in clinical use (e.g. follitropin alfa and follitropin beta) are derived from Chinese hamster ovary cell lines (CHO). Previously, in a population pharmacokinetic analysis of first-in-human data after single ascending doses [2], body weight was identified as a factor that negatively correlates with serum FE 999049 concentration. In the present work the FE 999049 population pharmacokinetics after multiple dosing are characterised. In addition, the endogenous FSH contribution to the total FSH levels and the covariate influence of other reproductive hormones are evaluated.

2. Methods

2.1 Clinical Trial Design and Data

Data was generated in a randomised, double-blind, active control, multiple dose trial with the aim to investigate the safety, tolerability, immunogenicity, pharmacokinetics, and pharmacodynamics of FE 999049

in healthy women. The trial was performed according to the Helsinki declaration and good clinical practice. It was approved by regulatory authorities and local ethics committees. All subjects gave written informed consent to participate. The trial has been described in more detail in a recent publication comparing the pharmacokinetic and pharmacodynamic properties of FE 999049 and GONAL-F (follitropin alfa, EMD Serono) using non-compartmental analysis (NCA) [3]. Briefly, 49 healthy women were given daily subcutaneous doses of 225 IU rFSH for 7 days. 24 out of the 49 women were treated with FE 999049 and 25 women received GONAL-F as an active comparator. Prior to the trial (day -28 and -14) subjects were given two doses of a GnRH agonist (LUPRON DEPOT, 1-month depot) to down-regulate endogenous FSH.

Blood samples for FSH, inhibin B, estradiol, progesterone, and LH measurements were collected 60 and 30 minutes prior to administration of FE 999049, immediately before administration, and once a day for 15 days. In addition, after administration of the last dose on day 6 and until day 8 the FSH concentration was measured every 4th hour. Analysis of serum FSH concentrations was performed at Ferring Pharmaceuticals A/S with a validated immunoassay based on electrochemiluminescence (MSD sectorTM Imager 2400) with a lower limit of quantification (LLOQ) of 0.075 μg/L.

The present analysis included data from the 24 women receiving FE 999049. Three out of the 672 FSH measurements (0.4 %) were below the quantification limit and excluded from further analysis. The personal demographics and baseline characteristics for the included subjects are listed in Table 1. Subjects with missing hormone baseline values were given the median population baseline value. Between day 6 and 8, FSH was measured every 4th hour and the other hormones were measured only once a day, leaving missing hormone values in between. To fill out the extra time points the last measured hormone values were carried forward.

2.2 Pharmacokinetic Modelling

The PK model was developed using nonlinear mixed effects modelling, where both the population parameters, interindividual variability (IIV), and residual errors are estimated. For parameters with IIV the i^{th} subject's individual parameter, θ_i, is log-normal distributed:

$$\theta_i = \theta \exp(\eta_i), \quad (1)$$

where θ is the typical population parameter and η_i is the individual random effect from an approximately normal distribution with mean zero and variance ω^2 for describing the IIV of the parameter. In the model potential influential factors can be tested for significance as a covariate to explain some of the IIV in a parameter. Thus the set of individual parameters, Θ_i, is given as a function of the typical population parameters, Θ, individual values of the covariates, $\boldsymbol{c}_i$, and random effects, $\boldsymbol{\eta}_i$:

$$\Theta_i = g(\Theta, \mathbf{c}_i, \boldsymbol{\eta}_i). \quad (2)$$

The residual errors, ε, are assumed normally distributed with mean zero and variance σ^2, and are the unexplained deviations of model predictions from the observations:

$$y_{ij} = f(t_{ij}, \Theta_i) + \varepsilon_{ij}. \quad (3)$$

y_{ij} is the observation at time t_{ij} and the subscript ij denotes the j^{th} number for subject i. The individual model prediction $f(\cdot)$ at time t_{ij} is calculated from the individual parameters, Θ_i.

Model development was guided by changes in the NONMEM objective function value (OFV), precision of parameter estimates, and graphical model goodness-of-fit assessments including visual predictive checks (VPC). In the VPC the observed data is compared to model predictions based on 1000 simulated trial datasets using the final model. It displays the observations and the 2.5th, 50th, and 97.5th percentiles of observations and the 95% confidence intervals (CI) for the corresponding model predictions are plotted. The OFV is approximately proportional to -2log likelihood. The difference in OFV between two nested models is approximately χ^2-distributed, with degrees of freedom (df) equal to the difference in the number of parameters. Based on this, the statistical significance for inclusion/exclusion of a model parameter can be judged. A significance level of 0.05 was used for discrimination among nested models and covariate testing. It corresponds to a 3.84 change in OFV for 1 df.

Body weight, age, and hormone (estradiol, inhibin B, progesterone) values at baseline were tested as potential covariates explaining some of the IIV in parameter estimates. Inhibin B was also tested as a covariate over time, since it has a purely inhibitory effect at FSH. Besides a decrease in OFV the significance of a covariate was also evaluated by looking at the reduction in the IIV measured as coefficient of variation (CV) for the parameter's random effect.

2.3 Software

The models were implemented and parameters estimated in NONMEM 7.2.0 (Icon Development Solutions, USA) [4]. Data handling and graphical representations were performed in R version 2.11.1 [5]. VPCs were performed using PsN [6;7] and plotted using Xpose [8].

3. Results

Measurable FSH levels before drug administration indicated that endogenous FSH was not fully suppressed in this trial. To obtain accurate PK parameter estimates it was therefore necessary to model the total FSH at time *t* as the sum of the endogenous FSH and the exogenously administered rhFSH:

$$\mathrm{FSH}(t) = \mathrm{FSH}_{\mathrm{en}}(t) + \mathrm{rhFSH}(t). \tag{4}$$

The FE 999049 absorption was found to be delayed and a transit compartment was introduced with absorption rate k_{tr}. The change over time in the FE 999049 amount in the central compartment was given by

$$\frac{d\mathrm{rh}\quad(t)}{dt} = k_a\ \ \mathrm{rh}\quad\ _T(t) - k \cdot \mathrm{rh}\quad(t), \tag{5}$$

where $\mathrm{rhFSH}_T(t)$ is the amount entering from the transit compartment with rate constant k_a. The elimination rate constant k is given by clearance (CL) divided by volume of distribution (V). Since data is obtained after subcutaneous dosing the bioavailability (F) is not known. The CL and V estimated here are therefore the apparent clearance (CL/F) and apparent volume of distribution (V/F). The endogenous FSH was assumed to have the same elimination rate constant from the central compartment as FE 999049 and a zero order production rate constant k_{in}. The change in endogenous FSH amount over time in the central compartment was described as

$$\frac{d\mathrm{FSH}_{\mathrm{en}}(t)}{dt} = k_{\mathrm{in}} - k \cdot \mathrm{FSH}_{\mathrm{en}}(t). \tag{6}$$

The endogenous baseline FSH concentration ($\mathrm{FSH}_{\mathrm{bl}}$) was estimated for each subject to be the initial concentration in the central compartment before dosing. IIV was introduced for CL/F, V/F, k_{tr}, and $\mathrm{FSH}_{\mathrm{bl}}$. The variances of the IIV on CL/F and V/F were positively correlated. Body weight was an allometrically scaled covariate at CL/F and V/F with the power exponents fixed to 0.75 and 1, respectively. Adding body weight as a covariate reduced CV for the unexplained IIV from 18.1 to 15.6% CV for CL/F and from 22.0 to 18.4% CV for V/F.

The measured hormone levels were assumed to not affect the rFSH concentration but only tested as covariates on the endogenous baseline FSH parameter ($\mathrm{FSH}_{\mathrm{bl}}$). A trend towards lower estimated FSH baseline levels were seen in individuals with higher progesterone baseline levels (Figure 1). The progesterone baseline effect was confirmed to be a statistically significant covariate when modelled as an

inhibitory power function at the FSH_{bl} parameter. This decreased IIV in FSH_{bl} from 32.6 to 27.8% CV. Moreover, observed inhibin B levels (InhB(t)) suppressed endogenous FSH production rate (k_{in}) over time when introduced in the model as a time-varying covariate. An I_{max} function with a parameter $InhB_{ef}$ for the inhibin B concentration yielding half of maximum suppression was found to best describe the relationship. The equation for the total FSH amount then becomes

$$\frac{d\mathrm{FSH}(t)}{dt} = k_{\mathrm{in}} \cdot \left(1 - \frac{\mathrm{InhB}(t)}{\mathrm{InhB}(t) + \mathrm{InhB}_{\mathrm{ef}}}\right) + k_a \cdot \mathrm{rhFSH}_{TR}(t) - k \cdot \mathrm{FSH}(t). \quad (7)$$

Individual profiles for the resulting model predicted endogenous FSH level and observed inhibin B are shown in Figure 2. Accounting for the inhibin B suppression of the endogenous FSH contribution in this way increased the V/F estimate from 18.9 to 24.3 L. Not including inhibin B as a covariate in the final model resulted in an increase from 83.4 to 164.9% CV at k_{tr}.

When the best model was found in a forward development process it was checked if all the elements still were significant or if the model could be reduced. In Table 2 is listed the resulting increase in OFV from removing the elements. No reduction was possible. The best structural model is illustrated in Figure 3. A combined additive and proportional error model was used to describe the residual error. The final model parameter estimates are displayed in Table 3. The VPC plot (Figure 4) based on 1000 simulations, shows that the model predicts the FSH concentration PK profile well with appropriate variation.

4. Discussion

The FE 999049 population pharmacokinetics have previously been characterised after single dose administration [2]. In accordance with those results, the current analysis showed that after multiple FE 999049 dosing the population pharmacokinetics were also best described by a one-compartment model with first order elimination and absorption through a transit compartment. There was a correlation between CL/F and V/F, and body weight was an allometrically scaled covariate for both CL/F and V/F. Hereby, it was confirmed that lower average steady-state drug exposure in females correlates with higher body weight after repeated doses, which is in agreement with single dose FE 999049 results.

In contrast to the single ascending dose trial the FSH down-regulation in this trial was not complete, since a measurable endogenous FSH level was detected before any drug administration. The observed endogenous FSH contributes to total FSH measurements and thereby affects the evaluation of rhFSH concentrations in the trial, thus it had to be accounted for in the model. To adjust the model accordingly it was necessary to add an endogenous supply of FSH to the central compartment for proper prediction of total FSH concentration.

When evaluating the pharmacokinetics of drugs that are naturally occurring substances, data is often baseline corrected in order to get values only representing the exogenously administered drug. However, when dealing with hormones there may be fluctuations from the endogenous baseline value over time due to various feedback control systems. Even with gonadotropin down-regulation, the endogenous FSH level will most likely change over time as progesterone, inhibin B, and estradiol exert inhibitory and stimulatory feedback loops at the gonadotropin production and release. The potential impact of varying endogenous FSH levels at the pharmacokinetic assessment of FE 999049 was tested by incorporating different covariate relationships in the model. Before any drug was administered there was a tendency towards a decreased endogenous FSH baseline with increasing progesterone baseline. A power function for the relation between progesterone baseline and FSH_{bl} was shown to be significantly better than using a linear, an exponential, or an I_{max} function. When introduced as a time-varying covariate inhibin B was found to suppress the endogenous FSH production rate over time with an I_{max} function being the most significant relationship. When accounting for the inhibitory effect of inhibin B over time, the resulting model-predicted endogenous FSH concentration profiles indicated that the observed FSH baseline is an overestimate of the endogenous FSH level at all other time points than zero (Figure 2).

When multiple covariates and correlations have been added to the model, it is possible that initial significant relations have become redundant and hence can be removed from the model. It was therefore checked if the model could be reduced. Removing any relations resulted in an increase in OFV, thus all relations were still significant. Removing WT from both CL/F and V/F increased OFV less than removing only one of them. This could be due to that there is a correlation between the two parameters.

The population PK parameters estimated with nonlinear mixed effect modelling for single dose and multiple doses of FE 999049 are similar. In the modelling of the first in human data CL/F and V/F for a 65 kg woman was found to be 0.430 L/h and 28.0 L, respectively [2]. In this trial CL/F was 0.423 L/h and V/F was 24.3 L. By incorporating the inhibin B dynamics in the model and suppressing the endogenous FSH contribution instead of letting the FSH baseline be constant throughout the trial, V/F increased from 18.9 to 24.3 L. This suggests that without proper estimation of endogenous FSH levels over time a bias in V/F may be obtained.

5. Conclusion

The multiple dose FE 999049 population pharmacokinetics were in agreement with results obtained after single dose administration [2]. It was confirmed that after repeated drug administration, drug exposure also appears lower in females with higher body weight.

When subjects do not have fully suppressed endogenous FSH levels, it affects total FSH concentration, thus inclusion of endogenous FSH levels in the PK modelling of FSH preparations could be important. In

addition, the endogenous FSH level will possibly not be constant over time and quantifying the influence of the endocrine hormone dynamics during the trial may well be essential for proper estimation of pharmacokinetic parameters. The standard method of baseline correcting data does not account for a variation over time and could potentially cause an underestimation of serum drug concentration and hence inaccurate parameter estimation.

To better explore and link the time-varying impact of inhibin B on the endogenous FSH levels a more mechanistic modelling approach may be warranted for including indirect delayed response and hormone feedback mechanisms in a PKPD model and simultaneously quantify the inhibin B response to rFSH stimulation.

Compliance with Ethical Standards

Funding: This work was supported by Innovation Fund Denmark (Industrial PhD case number 11-117436).

Conflicts of Interest: Trine Høyer Rose, Daniel Röshammar, Lars Erichsen, and Lars Grundemar are all current or former employees at Ferring Pharmaceuticals A/S.

Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent: Informed consent was obtained from all individual participants included in the study.

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Table 1. Summary of subject characteristics

	Mean	(range)
Age (years)	31.1	(21.5 - 38.7)
Height (cm)	163.5	(149.0 - 175.3)
Weight (kg)	71.5	(46.1 - 86.6)
BMI (kg/m^2)	26.6	(20.8 - 28.9)
FSH (µg/L)	0.211	(0.089 - 0.376)
E2 (pg/mL)	24.21	(7.0 - 55.0)
LH (IU/L)	0.719	(0.1 - 1.4)
Prog (µg/L)	0.652	(0.08 - 1.49)
InhB (pg/mL)	22.0	(4.0 - 89.0)

Demographics and baseline hormone levels for the 24 subjects receiving FE999049. The values are mean with range in brackets. BMI is body mass index. FSH, E2, LH, Prog, and InhB is the measured baseline concentration of follicle stimulating hormone, estradiol, luteinizing hormone, progesterone, and inhibin B, respectively.

Table 2. Change in objective function value for reduced models

Removing	df	dOFV
WT at V/F	0	14.36
WT at CL/F	0	12.61
WT at CL/F and V/F	0	8.91
cov(CL/F,V/F)	1	11.77
Progesterone effect	1	7.01
Inhibin B effect	1	78.01

The resulting change in objective function value (dOFV) when removing covariates or the correlation between CL/F and V/F (cov(CL/F,V/F)). F: bioavailability, CL/F: apparent clearance, V/F: apparent volume of distribution, WT: body weight, df: degrees of freedom.

Table 3. Pharmacokinetic parameter estimates

Parameter	Estimate	(RSE%)	IIV CV%	(RSE%)	Shrinkage (%)
CL/F (L/h)	0.423	(3.9)	15.6	(12.7)	-1.05
V/F (L)	24.30	(4.6)	18.4	(14.4)	2.80
k_{tr} (h^{-1})	0.329	(17.0)	83.4	(24.1)	24.6
k_a (h^{-1})	0.148	(13.2)			
FSH_{bl} (µg/L)	0.162	(9.1)	27.8	(16.5)	5.51
$Progbl_{ef}$	-0.246	(34.7)			
$InhB_{ef}$	100	(37.2)			

Typical population parameter estimates obtained from modelling with the relative standard error (RSE) in brackets. F is the bioavailability and the parameters are the apparent clearance (CL/F), apparent volume of distribution (V/F), absorption rate from the dosing site (k_{tr}), absorption rate from the transit compartment (k_a), endogenous FSH baseline (FSH_{bl}), power exponent for progesterone baseline covariate effect ($Progbl_{ef}$), and inhibin B time-varying covariate effect ($InhB_{ef}$). For CL/F and V/F the value is the typical value for a woman weighing 65 kg. The interindividual variability (IIV) is listed as the percentage coefficient of variation (CV) with RSE in brackets and corresponding ETA shrinkage in percentage.

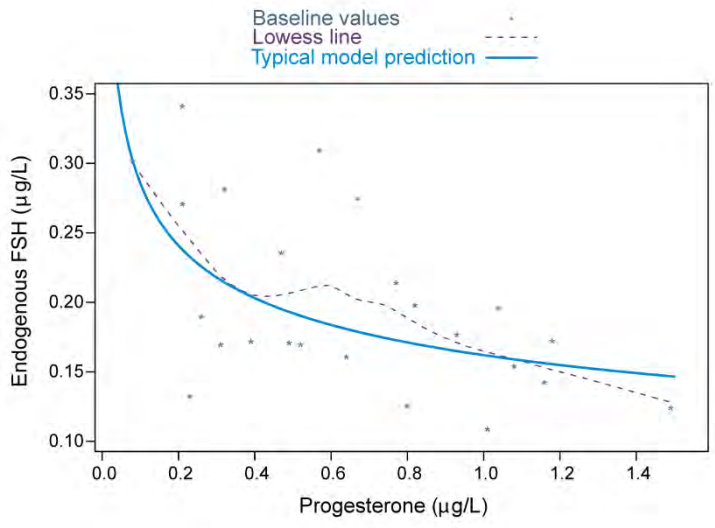

Figure 1 The relationship between endogenous FSH and progesterone at baseline. Points are individual predicted endogenous FSH baseline values (FSH_{bl}) and observed progesterone baseline values with a smooth lowess trend line (broken line). The solid line is the power function used in the model describing the typical population relationship for the effect of progesterone baseline at the parameter FSH_{bl}.

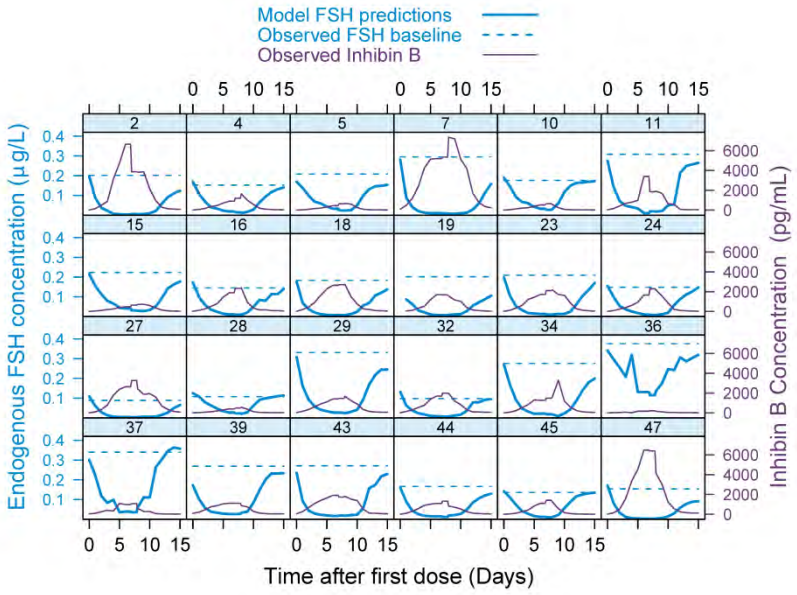

Figure 2 Individual hormone concentration profiles over time. The broken blue line is the level for the observed endogenous FSH baseline. The solid blue line is the model predicted endogenous FSH level when not assuming it to be constant throughout the trial but predicting the level based on what the observed inhibin B levels (purple line) over time are based on the model. The number at each subplot is the subject ID number.

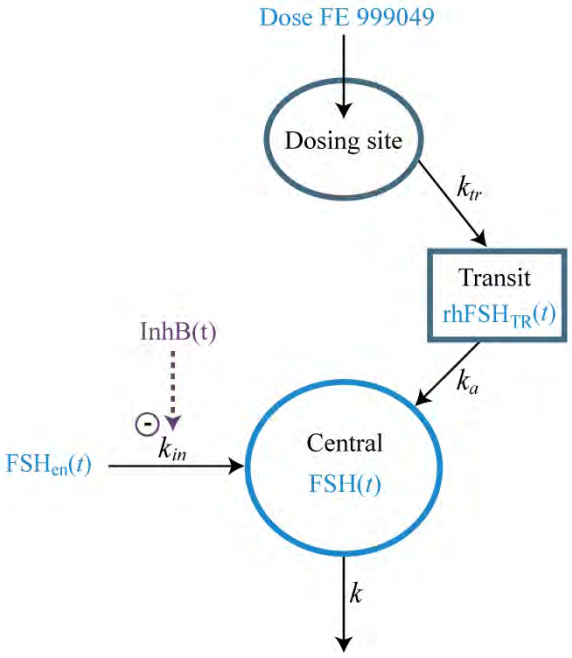

Figure 3 A compartment diagram showing the pharmacokinetic model for FE 999049. It illustrates the contribution of endogenous FSH ($FSH_{en}(t)$) to the central compartment with a production rate k_{in} being suppressed by inhibin B levels (InhB(t)) over time. FE 999049 after administration is absorbed from the dosing site with a rate k_{tr} to a transit compartment in which the amount is $rhFSH_T(t)$. The absorption rate from the transit compartment to the central compartment is k_a, where total FSH(t) is measured without differing between the sources. From here the elimination rate for the total FSH is k.

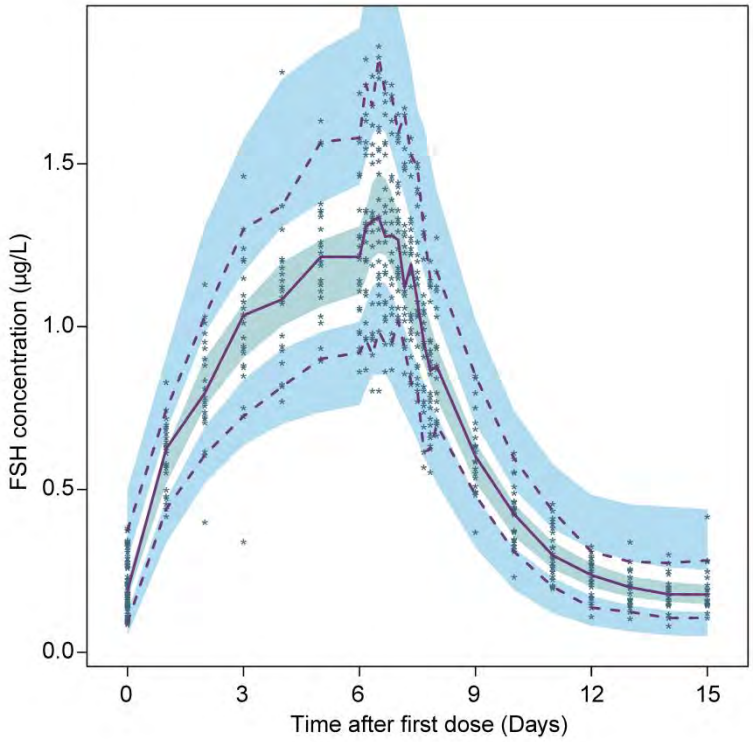

Figure 4 Visual predictive check for the final model. It shows the individual observed FSH concentrations (points) and the 2.5th, 50th, and 97.5th percentiles of observations (lines). The shaded areas are the 95% confidence intervals for the 2.5th, 50th, and 97.5th percentiles of the simulations.

Paper III

Semi-Mechanistic Pharmacokinetic-Pharmacodynamic Modelling of Inhibin B Levels after Multiple Doses of the Recombinant Human Follicle Stimulating Hormone FE 999049 in Infertile Women

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Abstract

Objectives: To develop a semi-mechanistic population pharmacokinetic-pharmacodynamic (PKPD) model describing the relationship between follicle stimulating hormone (FSH) and inhibin B in infertile women receiving multiple dosing of FE 999049 - a recombinant human FSH (rhFSH).

Methods: Data from a FE 999049 phase 2 multiple dose trial including 222 infertile women was analysed using non-linear mixed effect modelling in NONMEM 7.2.0. The patients received daily subcutaneous doses of either 5.2, 6.9, 8.6, 10.3, or 12.1 µg FE 999049 for a maximum of 16 days. Data contained 1160 FSH measurements and 1155 inhibin B measurements. The FE 999049 concentration-time profile and inhibin B levels were modelled simultaneously in a PKPD model taken the dynamics of the reproductive hormone system into consideration.

Results: The FE 999049 population pharmacokinetics were described by a one compartment distribution model with a transit compartment for a delayed absorption. The total measured FSH concentration consisted of both an endogenous FSH contribution and the exogenously administered rhFSH (FE 999049). The total FSH level stimulated the inhibin B production rate in an indirect turnover response model, and inhibin B level simultaneously exerted a negative feedback loop at the endogenous FSH production. Body weight was a significant covariate in the model which resulted in lower FSH exposure as well as lower inhibin B response with higher body weight.

Conclusion: The semi-mechanistic PKPD model can be used to evaluate longitudinal FSH dose-exposure-inhibin B relationship over time as an early marker of response in clinical infertility studies. A decrease in inhibin B response is also seen with increasing body weight in the same way as FSH exposure is affected by body weight.

Introduction

FE 999049 is a novel recombinant human FSH (rhFSH, Ferring Pharmaceuticals A/S) expressed in a cell-line of human fetal retinal origin (PER.C6®, Crucell, Leiden, The Netherlands). FE 999049 is intended for controlled ovarian stimulation by subcutaneous administration to induce maturation of multiple follicles for in vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSI) treatment. Hormone therapy with exogenous administration of follicle stimulating hormone (FSH) can be used for infertility treatment when the cause is not ovarian failure and the ovaries are still responsive to stimulation and contain functional primordial follicles. This is the case for female ovulation disorders and unexplained infertility which accounts for 40-50% of infertility cases[1, 2].

FSH is a gonadotropin produced by the anterior pituitary gland upon stimulation by gonadotropin releasing hormone (GnRH) from the hypothalamus. FSH is a key hormone in female reproductive function as it stimulates the ovaries to induce follicular development and hormone production. One of the ovarian hormones produced is inhibin B which exerts a negative feedback loop at the FSH production. Inhibin B has been identified as the earliest measureable hormone marker of response to gonadotropin treatment[3, 4] and there is substantial evidence that suggests inhibin B is a promising predictor for ovarian response. Inhibin B baseline and the rise in inhibin B after gonadotropin administration are higher in good responders[5, 6]. Number of oocytes retrieved correlates with inhibin B levels and change in inhibin B during treatment[3, 7, 8]. Furthermore, inhibin B correlates with both oocyte quality and number of eggs fertilised[9], total follicular volume[4], and antral follicle count[8].

It could therefore be valuable to predict and follow the inhibin B response throughout treatment. The population pharmacokinetics of FE 999049 have recently been described after single and repeated administration (paper I and II). The objective of the current analysis was to develop a semi-mechanistic population pharmacokinetic-pharmacodynamic (PKPD) model simultaneously describing the dynamics between endogenous FSH, exogenous rhFSH, and inhibin B in patients receiving repeated administration of FE 999049 in a phase 2b dose finding trial. Body weight has been identified to be an important patient specific factor influencing the population PK of FE 999049 (paper I and II). Subjects with high body weight has in general been reported to experience lower drug exposure compared to subjects with low body weight following administration of the same dose. An additional objective of this paper was to explore how the body weight related differences in drug exposure translates into between-patient differences in the pharmacodynamic response to FSH treatment measured as the biomarker inhibin B.

Methods

Clinical Trial Design and Data

The data used for the analysis was collected in a phase 2b dose finding trial. It was a randomised, controlled, assessor-blinded, parallel-group, multicentre, multiple dose trial assessing the dose-response relationship of FE 999049 in women undergoing IVF/ICSI. The trial was performed according to the ethical principles in the Helsinki declaration and in compliance with good clinical practice and regulatory requirements. The trial was approved by local regulatory authorities and independent ethics committees. All subjects gave written informed consent to participate.

The trial has been described more in detail in an earlier publication[10]. In summary, 265 women with tubal infertility, unexplained infertility, infertility related to endometriosis stage I/II, or with partners diagnosed with male factor infertility were included in the trial. They received daily subcutaneous doses of either 5.2 (n=42), 6.9 (n=45), 8.6 (n=44), 10.3 (n=45), or 12.1 µg (n=46) (90, 120, 150, 180 or 210 IU) FE 999049 or 11 µg (n=43) (150 IU) GONAL- F (follitropin alfa, EMD Serono) for a maximum of 16 days. Randomisation was stratified according to antimüllerian hormone (AMH) level at screening (low: 5.0-14.9 pmol/L and high: 15.0-44.9 pmol/L). From day 5 after the first dose and throughout the stimulation period a GnRH antagonist (0.25 mg ganirelix acetate, ORGALUTRAN, MSD / Schering-Plough) was given to prevent a premature luteinising hormone (LH) surge.

Blood samples for FSH, LH, inhibin A, inhibin B, estradiol, progesterone, and testosterone measurements were collected immediately before the first administration, at day 3 and day 5 after the first dose, and hereafter at least every second day. When 3 follicles of ≥15 mm were observed, visits had to be performed on a daily basis. Each patient's treatment length depended on the individual response. Doses were given until three or more follicles with a diameter ≥17 mm were observed. The cycle would be cancelled if there were either too many (more than 35 follicles ≥12 mm) or too few (less than three follicles ≥10 mm at day 10) growing follicles.

Analysis of serum FSH concentrations was performed at ICON Central Laboratories, Dublin, Ireland, with a chemiluminescent immunometric assay (IMMULITE 2500 FSH (ROCHE), with a lower limit of quantification (LLOQ) of 0.0052 µg/L. Inhibin B was measured by an enzyme linked immunosorbent assay (Gen II ELISA (Beckman Coulter)) with an LLOQ of 4.8 pg/mL.

The current analysis included data from the 222 women receiving FE 999049 giving a total of FSH 1160 measurements and 1155 inhibin B measurements. The personal demographics for the included subjects are listed in Table 1.

Population PKPD Modelling

The population PKPD model was developed using a nonlinear mixed effects modelling. The population PK of FSH and the exposure-response relationship between FSH and inhibin B were described in a simultaneous PKPD model developed by a semi-mechanistic modelling approach in order to incorporate the hormone dynamics with feedback mechanisms in the longitudinal data.

Endogenous FSH baseline levels were detectable and had to be included in the model as a contribution to the central compartment such that total FSH concentration was modelled as a sum of endogenous FSH and exogenous administered rhFSH. Following the dynamics in the reproductive endocrine system total FSH should stimulation the production of inhibin B, and endogenous FSH production should be inhibited by inhibin B. Different functions were tested for describing the stimulating and inhibitory processes both with and without an effect compartment for a delayed indirect response. A GnRH antagonist was administered at day 5 to avoid a premature LH surge, but this also inhibits the production of FSH. To follow the protocol this inhibition of endogenous FSH after day 5 should be added in the model. Turnover models were used to describe the FSH and inhibin B concentrations.

When the dose is given subcutaneously, the bioavailability (F) is not known. The parameters estimated here are therefore the apparent clearance (CL/F) and apparent volume of distribution (V/F). In the current dataset there were few observations per individual, which was not sufficient to estimate all PK parameters, therefore V/F and the absorption rate constant (k_a) were fixed to the values found in the PK model from phase 1 data (paper II).

Individual parameters (θ_i) were obtained from typical population parameters (θ) with addition of an exponential function of an individual random effect (η_i) from an approximately normal distribution with mean zero and variance ω^2. Body weight was tested as a covariate by including individual body weight (WT_i) normalised to 65kg in a power function. Parameters with both IIV and body weight as a covariate were described by

$$\theta_i = \theta \exp(\eta_i) \left(\frac{\mathrm{WT}_i}{65\mathrm{kg}}\right)^{P_\theta}, \quad (1)$$

where P_θ is the power exponent for the weight effect at the parameter θ. The intraindividual variability was modelled with a combined additive and proportional residual error model. Data was log-transformed and separate error models for FSH and inhibin B were used.

Model Evaluation

The models were implemented and parameters estimated in NONMEM 7.2.0 (Icon Development Solutions, USA)[11]. Model development was guided by changes in the NONMEM objective function value (OFV), precision of parameter estimates, and graphical model goodness-of-fit assessments. The OFV is approximately proportional to -2log likelihood. The difference in OFV between two nested models is approximately χ^2-distributed, with degrees of freedom (df) equal to the difference in the number of parameters. Based on this, the statistical significance for inclusion/exclusion of a model parameter can be judged. A significance level of 0.05 was used for discrimination among nested models and covariate testing. It corresponds to a 3.84 change in OFV for 1 df. Besides a decrease in OFV the significance of a covariate was also evaluated by looking at the reduction in the IIV measured as coefficient of variation (CV) for the parameter's random effect.

Graphical evaluations include population mean concentration-time profiles, individual profiles, residual plots, and visual predictive checks (VPC). In the VPC the observed data is compared to model predictions based on 1000 simulated trial datasets using the final model. It displays the observations and the 2.5th, 50th, and 97.5th percentiles of observations as well as the 95% confidence intervals (CI) for the corresponding model predictions.

Data handling and graphical representations were performed in R version 2.11.1[12]. VPCs were performed using PsN[13, 14] and plotted using Xpose[15].

Results

The PK part of the model was found to be a one-compartment distribution model where the total amount of FSH was modelled as a sum of the endogenous FSH and the administered rhFSH in form of FE 999049. The absorption of FE 999049 was found to be first order with rate k_{tr} to a transit compartment from where it entered the central compartment with rate constant k_a. The elimination rate constant k is given by CL/F divided by V/F and was assumed to be the same for endogenous FSH and FE 999049.

An inhibitory effect from the GnRH antagonist at the endogenous FSH production rate constant (k_{endo}) were added after day 5 to be in accordance with the trial design. An exponential function, a linear function, log-linear, and several types of E_{max} models were tested for describing the FSH stimulation of inhibin B, but either the fit was poor or data did not support parameter estimation of the more advanced functions. A power function with the FSH concentration input normalised with baseline values and power exponent λ was found to describe data best.

It was tested if an effect compartment could be included to induce an indirect delayed response. There were indications of an improved fit hereby but the OFV was not significant better hence the effect compartment was left out. Likewise was tested several functions for the inhibition of endogenous FSH by the predicted inhibin B concentrations where an I_{max} function was found most suitable with the best OFV. When assuming full suppression is possible the I_{max} function is given by

$$\mathrm{I_{max}}(t) = 1 - \frac{\mathrm{InhB}(t)}{\mathrm{InhB}(t) + \mathrm{IC_{50}}}$$

where IC_{50} is the inhibin B concentration giving half suppression. The structural model was described by the differential equations (2)-(5), one for each of the four compartments in the model as illustrated in Figure 1.

$$\frac{d\mathrm{rhFSH}_{DS}(t)}{dt} = -k_{tr} \cdot \mathrm{rhFSH}_{DS}(t) \quad (2)$$

$$\frac{d\mathrm{rhFSH}_{TR}(t)}{dt} = k_{tr} \cdot \mathrm{rhFSH}_{DS}(t) - k_a \cdot \mathrm{rhFSH}_{TR}(t) \quad (3)$$

$$\frac{d\mathrm{FSH}(t)}{dt} = k_{\mathrm{endo}} \cdot (1 - \mathrm{ANTA_{ef}}) \cdot \left(1 - \frac{\mathrm{InhB}(t)}{\mathrm{InhB}(t) + \mathrm{IC_{50}}}\right) + k_a \cdot \mathrm{rhFSH}_{TR}(t) - k \cdot \mathrm{FSH}(t) \quad (4)$$

$$\frac{d\mathrm{InhB}(t)}{dt} = k_{\mathrm{in}} \cdot \left(\frac{\mathrm{FSH}(t)/(\mathrm{V/F})}{\mathrm{FSH_{bl}}}\right)^{\lambda} - k_{\mathrm{out}} \cdot \mathrm{InhB}(t) \quad (5)$$

$\mathrm{ANTA_{ef}}$ is the effect of the GnRH antagonist and is given by

$$\mathrm{ANTA_{ef}} = \begin{cases} 0 & , \quad t \leq 5 \\ \dfrac{\mathrm{GnRH_{anta}}}{1 + \mathrm{GnRH_{anta}}} & , \quad t > 5 \end{cases}$$

$\mathrm{rhFSH_{DS}}(t)$ and $\mathrm{rhFSH_{TR}}(t)$ is the FE 999049 amount left at the dosing site and in the transit compartment at time t in days, respectively. FSH(t) is the total amount of FSH in the central compartment, thus the FSH concentration is obtained by dividing the amount with V/F. InhB(t) is the inhibin B concentration at time t. Inhibin B had a production rate constant k_{in} and was eliminated by the rate constant k_{out}. The endogenous FSH baseline concentration ($\mathrm{FSH_{bl}}$) and inhibin B baseline concentration ($\mathrm{InhB_{bl}}$) was estimated for each subject to be the initial concentration in the central compartment (equation (4)) and in the inhibin B compartment (equation (5)) before dosing, respectively.

A random effect for IIV was introduced for CL/F, k_{tr}, $\mathrm{FSH_{bl}}$, $\mathrm{InhB_{bl}}$, and λ. Choices of stimulation and inhibiting feedback functions greatly affected the other parameter estimates and a full covariance matrix

was therefore tested and found to be significant better. A combined additive and proportional error model was used to describe the residual error for FSH and inhibin B separately.

Body weight was an allometrically scaled covariate at CL/F and V/F with the power exponent P_θ in equation (1) fixed to 0.75 and 1, respectively. Adding body weight as a covariate reduced CV for the unexplained IIV from 27.2 to 23.1% CV for CL/F. Body weight was also found to be a significant covariate at k_{tr} and λ with a reduction from 51.1, and 48.9% CV to 48.3, and 42.7% CV, respectively.

The estimated model parameters are listed in Table 2. Mean concentration profiles are shown in Figure 2 and VPCs in Figure 3 with separate graphs for FSH and inhibin B for each dose. The mean predictions fit observations nicely but in the VPC an over-prediction for the lowest dose and an under-prediction for the highest dose is observed for FSH concentrations, and inhibin B is over-predicted for the highest dose. Since the frequency subjects come in to the clinic for measurements depends on fulfilment of pre-set criteria for follicle number and size, different number of subjects are measured per day. There are even days with only one subject from a dosing group and therefore the observed percentiles in the VPC collapse to the same value.

FSH and inhibin B concentration-time profiles were simulated for three subjects weighing 50 kg, 75 kg, and 100 kg, respectively, after 7 doses of 10 µg FE 999049 using the model with typical parameters (Figure 4). The FSH exposure as well as inhibin B response decrease with increasing body weight.

Discussion

The FE 999049 dose-concentration-inhibin B response relationship was described by a semi-mechanistic PKPD model with incorporation of endogenous FSH. The total FSH concentration stimulates the inhibin B production rate. In return the inhibin B levels suppress endogenous FSH production rate over time when modelled as a simultaneous negative feedback. The PK information in the data was very sparse and not enough to estimate all PK parameters, so k_a and V/F were fixed to values from paper II. In addition IC_{50} and k_{out} for inhibin B had to be fixed to stabilise the model.

The inclusion of a full covariance matrix indicates correlation between PK and PD parameters and significant variation between subjects. In the reproductive system FSH and inhibin B are correlated, thus it makes sense the model parameters describing the relationship are as well. Different functions for the stimulatory and inhibitory dynamics between FSH and inhibin B were tested both with and without an effect compartment. It could be necessary with an effect compartment but the current data could not support the estimation of an extra parameter for the effect compartment nor a more complex stimulation function.

De Greef et al.[16] described the FSH stimulation of inhibin B production by a sigmoidal E_{max} function in an indirect response model. However, even with a large dataset they had to fix the Hill coefficient due to a large standard error. In addition, it was a sequential model where a population PK model was

developed first and then the FSH concentrations were used in the PD model. It was mostly an empirical model and they did not account for endogenous FSH. Their model did not predict inhibin B levels well at first and to catch an undershoot in inhibin B below the baseline values at later time points, a hypothetical modulator that stimulated the elimination of inhibin B was included to further lower the predicted level. Together with three PD models for follicular volume, cancellation rate, and number of oocytes, they used the model to simulate ovarian response and dose selection.

Inhibin B levels have also been described by others after multiple s.c. doses of Gonal-F in pituitary down-regulated healthy female volunteers with a sequential PKPD model[4]. They did not use a population modelling approach but fitted the model to individual data. The PK model was previously described by an exponential equation with the same data[17] and the estimated PK parameters for each subject were fixed in the PKPD model. Subsequently, the PK model was linked with an effect compartment to the PD model in which the inhibin B response was calculated by a power function from the FSH concentrations in the effect compartment. A high interindividual variation in inhibin B response was observed but no covariate analysis were performed hence no factors causing the variation were identified. They found no correlation between FSH concentrations and inhibin B, and thus concluded that the high variation between subjects in PD parameters was not due to pharmacokinetic variations but different pharmacodynamic sensitivity. It is therefore not enough to adjust dose after variations in FSH concentrations but the response should also be taken into account. Another study used the same PKPD model with a new dataset[18] and confirmed the results. However, in that study there was a measurable endogenous FSH at baseline and a constant term was thus added to the power function in the PD model for the effect at baseline. In addition they suggested that variation in pharmacokinetics was caused by large fluctuations in endogenous FSH production over time.

In these previous models, inhibin B has been described with different methods and functions, but they were all sequential PKPD models. They too observed great variation in response and one group concluded that endogenous FSH matter. To our knowledge this work is the first PKPD model for FSH and inhibin B modelling the concentrations simultaneously with incorporation of change in endogenous FSH over time after exogenous FSH administration. Unfortunately, the data was not sufficient to properly describe the full dynamics. Furthermore, the data was unbalanced with few samples per subject, who in addition did not stay in the trial the same number of days. When this is not accounted for in the model, it can cause imprecise prediction for later time points, in particular for higher doses where subjects might sooner reach the follicle criteria.

In order to investigate the impact of body weight on FSH exposure and inhibin B response simulations were performed for three patients weighing 50 kg, 75 kg, and 100 kg who received 7 daily doses of FE 999049. The simulated FSH exposure decreased with increasing body weight in agreement with previous findings (paper I). After the last dose, the FSH exposure decrease to an endogenous level lower than the pre-dose level due to suppression by the GnRH antagonist and inhibin B levels. Since FSH stimulates the

inhibin B production the simulated inhibin B response also decrease with increasing body weight. In addition, body weight affects the FSH stimulation of inhibin B thus the extent of difference between the concentration-time profiles are different for inhibin B response compared to FSH exposure. Body weight play a role in the variation in exposure and thus response but other factors are also involved. Endogenous FSH and variation in its level over time is likely an influential factor too.

There are evidence of inhibin B being the first biomarker possible to measure in infertility treatment with FSH products and thus a predictor for follicle development in response to FSH[3, 4, 8]. Hence, it is useful to be able to predict inhibin B levels in a patient to determine if the dose is effective and antral follicles have been recruited and entered the gonadotropin dependent growth phase. The model from this work could be used in simulations to predict outcomes of different doses to women of different weights. Then, the potential of inhibin B as a marker for ovarian response could be investigated and be related to later clinical PD endpoints like oocytes retrieved. In addition, the model could be used to simulate the typical dose-response relationship for inhibin B to see what dose range is required to get a proper response in inhibin B. Using the model should increase precision compared to only looking at data with standard statistical methods or when using a simple empirical dose-response model, and can thus improve decision making and dose selection.

Declaration of Conflicting Interests

Trine Høyer Rose, Daniel Röshammar, Lars Erichsen, and Lars Grundemar are all current or former employees at Ferring Pharmaceuticals A/S.

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Dose (µg)	**5.18** n=42	**6.90** n=45	**8.63** n=44	**10.35** n=45	**12.08** n=46
Age (years)	33.6 (26-37)	32.3 (21-37)	32.8 (27-37)	32.3 (25-37)	32.6 (25-37)
Weight (kg)	62.4 (48.0-95.2)	63.0 (47.0-84.4)	62.8 (47.0-82.0)	60.7 (48.8-82.0)	60.9 (46.0-85.0)
BMI (kg/m^2)	23.0 (18.7-32.0)	23.2 (18.6-30.9)	23.2 (18.3-30.1)	22.5 (18.4-27.7)	22.2 (18.9-30.1)
FSH (µg/L)	0.35 (0.17-0.64)	0.37 (0.12-0.83)	0.37 (0.21-0.65)	0.37 (0.17-0.65)	0.42 (0.15-1.35)
InhB (pg/mL)	79.7 (8.7-161.8)	83.3 (6.0-132.5)	82.7 (19.0-169.4)	85.9 (29.6-143.0)	82.1 (9.2-153.9)

Table 1 Personal demographics for the 222 patients receiving FE 999049 used in the modelling. The values are mean with range in brackets for each of the 5 dose groups. n indicate the number of patients in each group. BMI is body mass index. FSH and InhB is the measured baseline concentration of follicle stimulating hormone and inhibin B, respectively.

Parameter	Estimate	(RSE%)	IIV CV%	(RSE%)
CL/F (L/h)	0.601	(2.2)	23.1	(7.4)
V/F (L)	24.3[a]			
k_{tr} (h^{-1})	0.0163	(5.0)	48.3	(11.4)
k_a (h^{-1})	0.148[a]			
FSH_{bl} (µg/L)	0.355	(2.4)	25.3	(5.3)
$InhB_{bl}$ (pg/mL)	79.8	(4.5)	35.9	(10.3)
IC_{50}	100[a]			
$GnRH_{anta}$	0.434	(14.7)		
k_{out}	0.7[b]			
λ	4.16	(4.6)	42.7	(10.5)
Power exponent P_θ for body weight at				
CL/F	0.75[c]			
V/F	1[c]			
k_{tr}	-1.40	(26.4)		
λ	1.43	(19.6)		

Table 2 Typical population parameter estimates obtained from modelling with the relative standard error (RSE) in brackets. The interindividual variability (IIV) is listed as the percentage coefficient of variation (CV) with RSE in brackets. F is the bioavailability and the parameters are the apparent clearance (CL/F), apparent volume of distribution (V/F), absorption rate from the dosing site (k_{tr}), absorption rate from the transit compartment (k_a), endogenous FSH baseline (FSH_{bl}), inhibin B baseline ($InhB_{bl}$), inhibin B concentration yielding half of maximum suppression (IC_{50}), suppressive effect of the gonadotropin releasing hormone antagonist ($GnRH_{anta}$), inhibin B elimination rate (k_{out}), and the power exponent for the FSH stimulation at inhibin B (λ). For CL/F, V/F, k_{tr}, and λ the value is the typical value for a woman weighing 65 kg and P_θ is the power exponent from equation (1) for the body weight effect at the parameters.

[a] fixed to values from paper II. [b] fixed value. [c] allometric values.

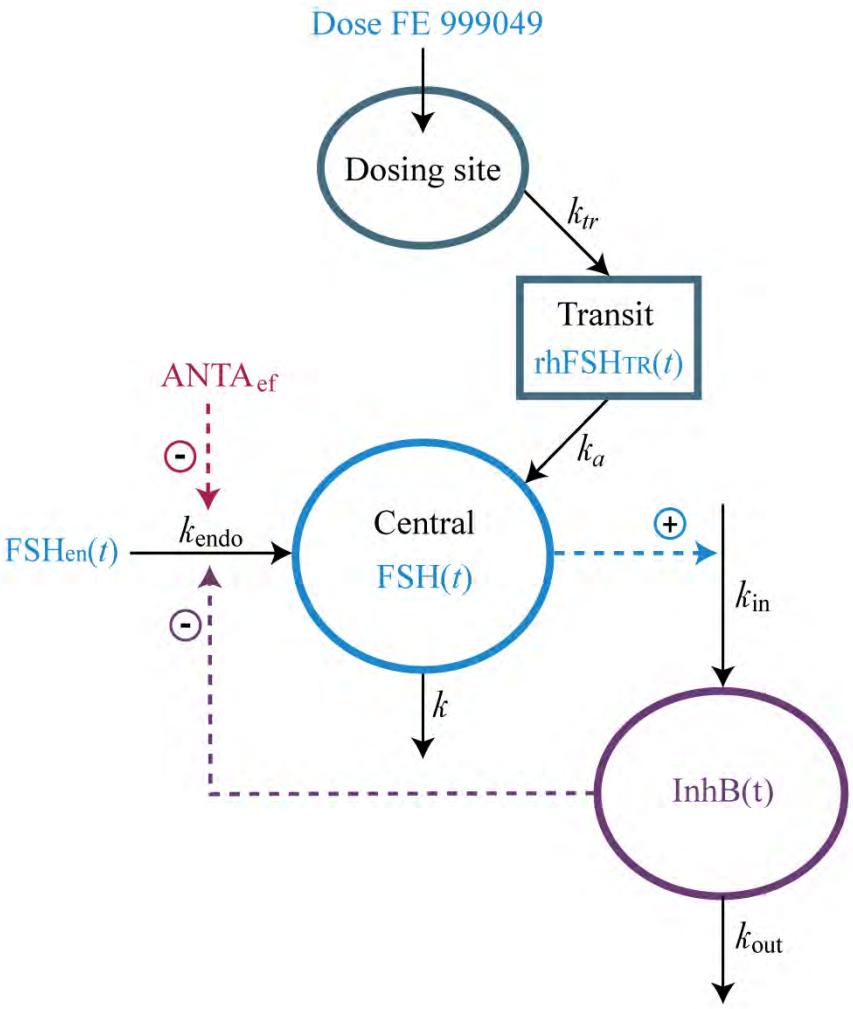

Figure 1 Compartment diagram of the PKPD model . Contributions to the total FSH amount in the central compartment (FSH(t)) are FE 999049 from the transit compartment ($rhFSH_{TR}(t)$) and endogenous FSH ($FSH_{en}(t)$). The endogenous FSH production rate (k_{endo}) is inhibited by predicted inhibin B concentrations (InhB(t)) and after day 5 also by a gonadotropin releasing hormone (GnRH) antagonist. The inhibin B production rate (k_{in}) is stimulated by FSH.

k_{tr}: rFSH absorption rate from the dosing site, k_a: rFSH absorption rate to the central compartment, k: FSH elimination rate from the central compartment, k_{out} inhibin B elimination rate.

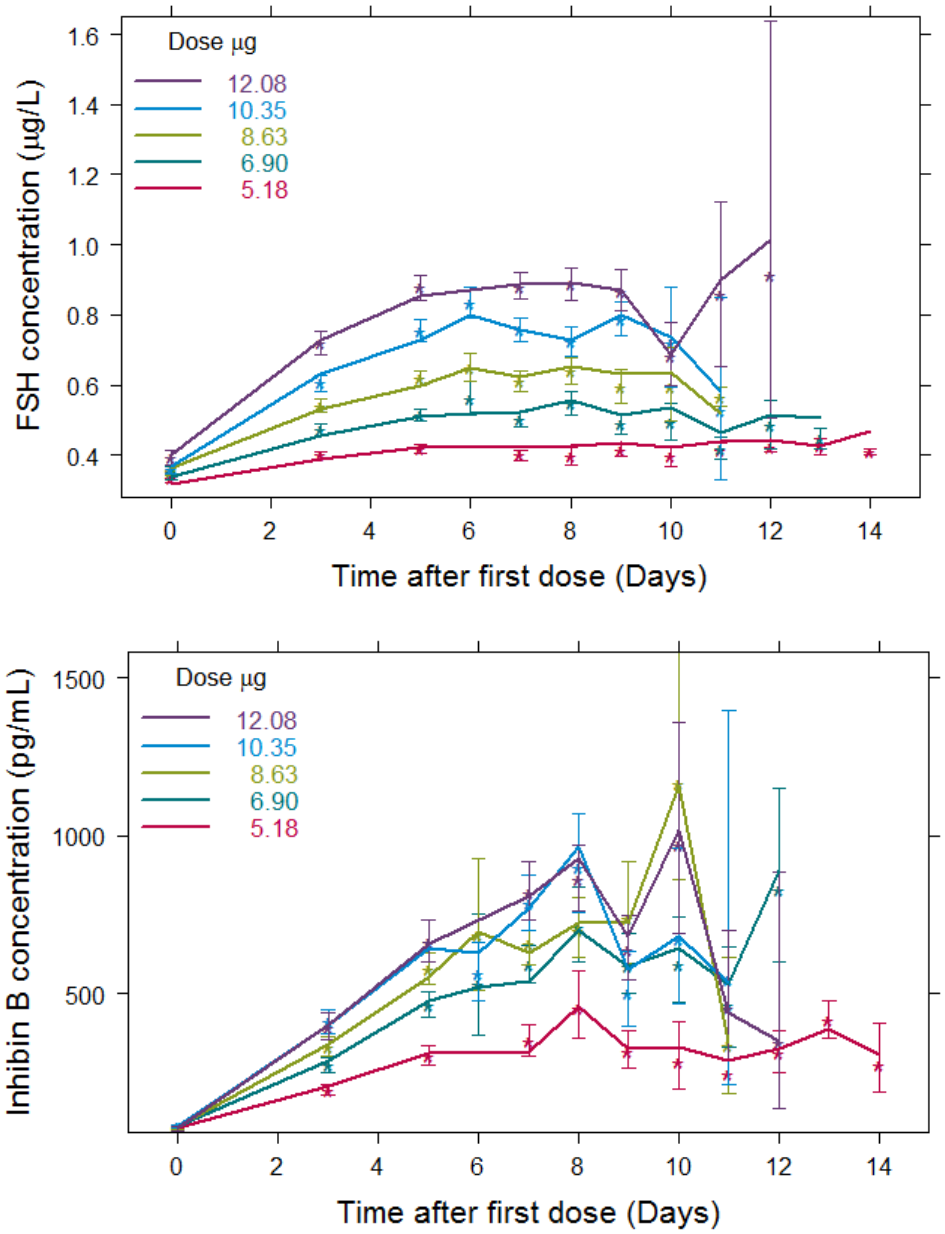

Figure 2 Illustration of observed FSH (top) and inhibin B (bottom) concentrations and model predictions for each treatment group. Points are mean of observations with standard error (SE) bars. Lines are typical model predictions.

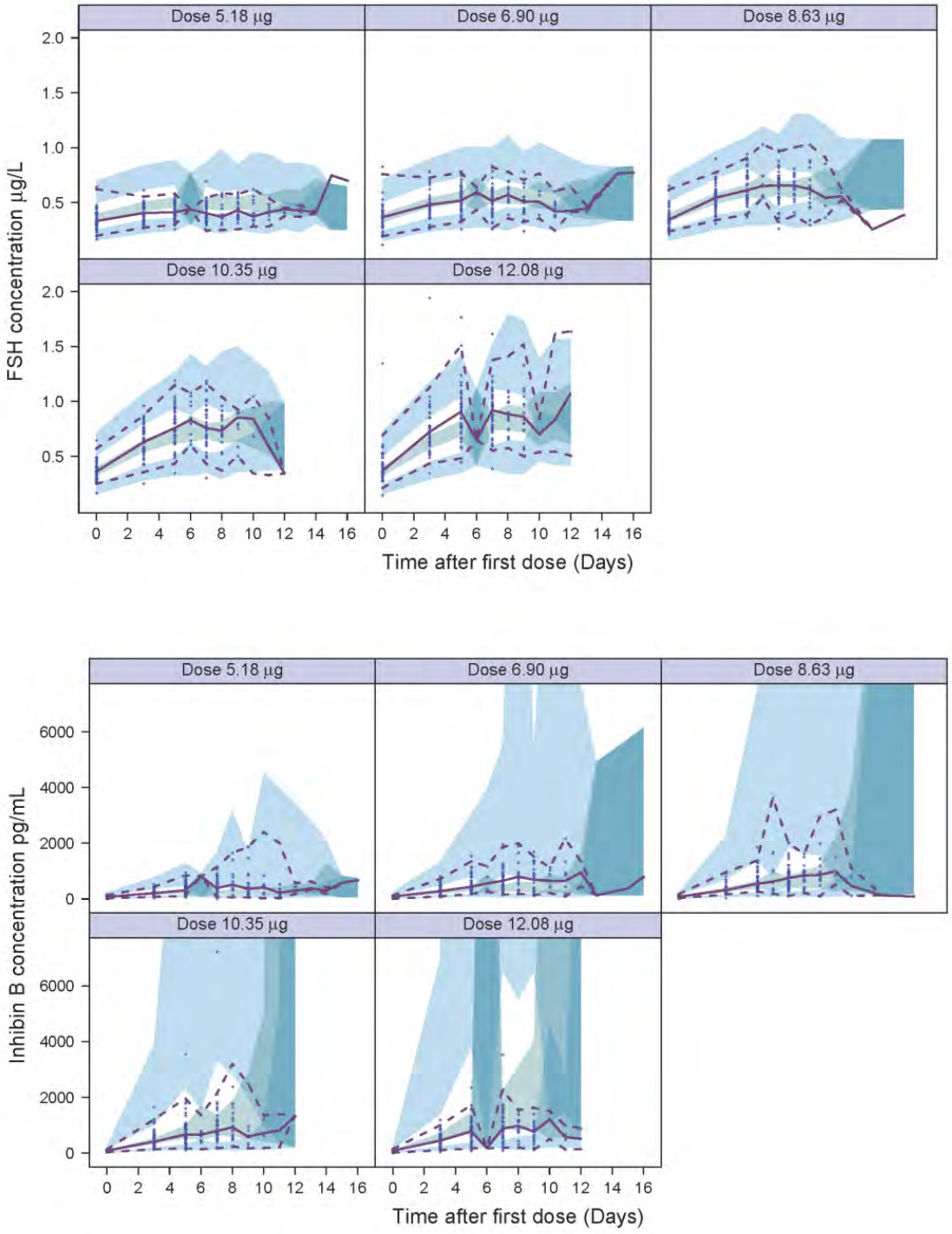

Figure 3 Visual predictive checks for FSH (top) and inhibin B (bottom). The points are observations with purple lines for the 2.5th, 50th, and 97.5th percentiles of observations. The shaded areas are the simulated 95% confidence intervals (CI) obtained with the model for the 2.5th, 50th, and 97.5th percentiles.

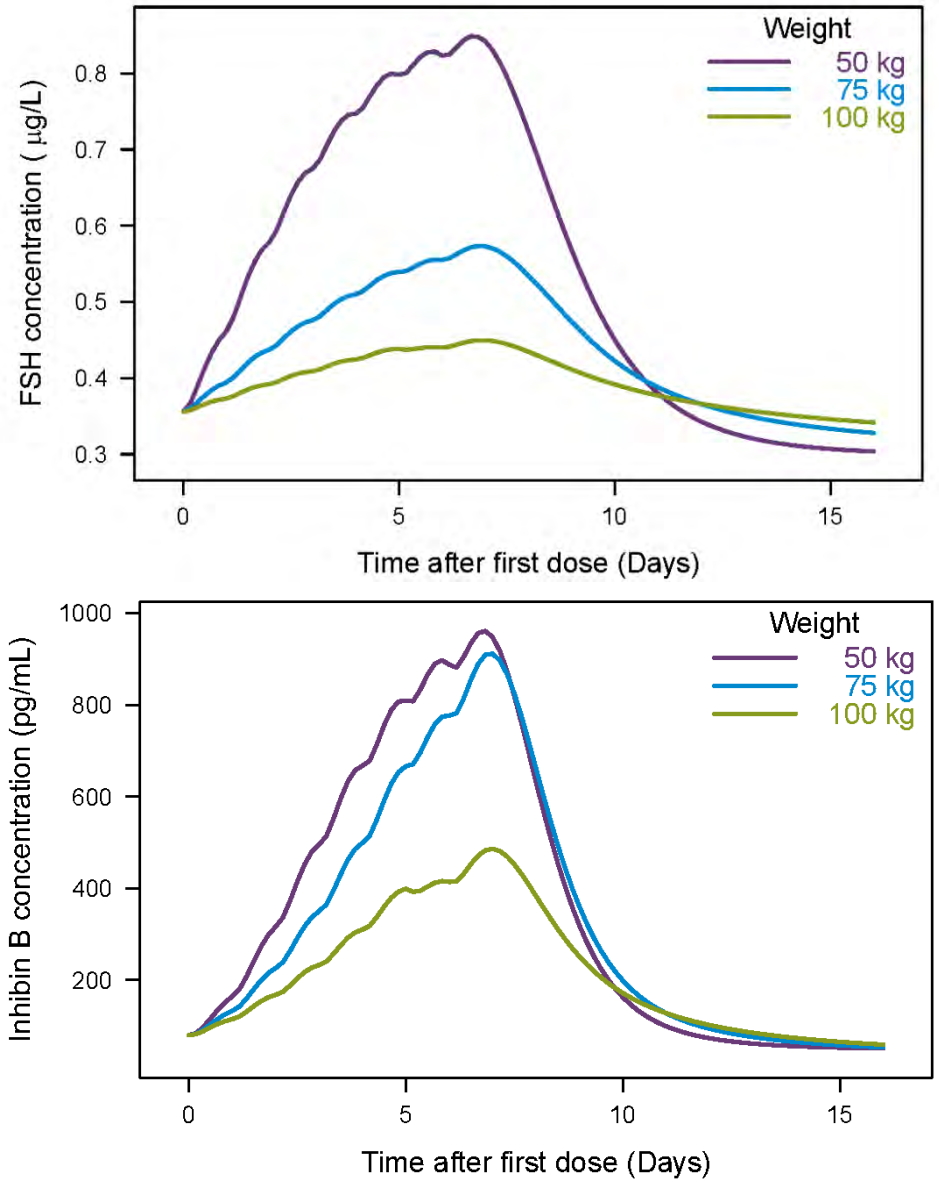

Figure 4 Illustration of simulated FSH (top) and inhibin B (bottom) concentrations for three patients of different weight. The patients received 7 daily doses of 10 µg FE 999049.

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