Post weaning altrenogest use in sows - Jessika van Leeuwen 2011

Post weaning altrenogest use in sows

Follicle growth, endocrine profiles and subsequent fertility

Jessika van Leeuwen

Post weaning altrenogest use in sows;

Follicle growth, endocrine profiles and subsequent fertility

Jessika van Leeuwen

Thesis committee

Thesis supervisor

Prof. dr. ir. B. Kemp
Professor of Adaptation Physiology
Wageningen University

Thesis co-supervisor

Dr. ir. N.M. Soede
Associate professor, Adaptation Physiology Group
Wageningen University

Other members

Prof. dr. ir. L.A. den Hartog, Wageningen University

Prof. dr. T.A.E. Stout, Utrecht University

Prof. dr. D. Maes, Ghent University, Belgium

Dr. R.V. Knox, University of Illinois, Illinois, USA

This research was conducted under the auspices of
the Graduate School of Wageningen Institute of Animal Science (WIAS).

Post weaning altrenogest use in sows;

Follicle growth, endocrine profiles and subsequent fertility

Jessika van Leeuwen

Thesis

Submitted in fulfillment of the requirements for the degree of doctor

at Wageningen University

by the authority of the Rector Magnificus

Prof. dr. M.J. Kropff,

in the presence of the

Thesis Committee appointed by the Academic Board

to be defended in public

on Friday 9th of September 2011

at 1.30 p.m. in the Aula.

Van Leeuwen, Jessika
Post weaning altrenogest use in sows – Follicle growth, endocrine profiles and subsequent fertility

PhD thesis, Wageningen University, the Netherlands (2011)
With references, with summaries in English and Dutch

ISBN: 978-90-8585-996-3

Voor Ankie, uit dankbaarheid

Para Juan, porque siempre tuvo fe.

Abstract

van Leeuwen, Jessika (2011). Altrenogest use in sows - Follicle growth, endocrine profiles and subsequent fertility. PhD thesis, Wageningen University, the Netherlands

A severe negative energy balance during first lactation may result in poor reproductive performance in the second litter. Allowing the sow recovery time after weaning by inseminating the sow the second estrus after weaning (skip a heat) improves reproductive performance. Postponing estrus for a shorter period after weaning using daily altrenogest administration has also been found to influence reproductive performance. The aim of this thesis was to develop a better understanding of consequences of altrenogest after weaning for follicle development and subsequent reproductive performance. Therefore, a first experiment investigated follicle development during and after post weaning altrenogest treatments and related this to subsequent fertility. It showed that follicle size increased during altrenogest treatment (independent of dose and duration), but no effects were found on fertility parameters on day 5 of gestation. Therefore, a second experiment studied the effect of duration of treatment on follicle development and subsequent farrowing rate and litter size. This experiment showed again an increase in follicle size and also showed that long altrenogest treatments (15 d) improve fertility, but that shorter treatments (8 d) reduce farrowing rates in sows with large follicles at weaning. So, to prevent outgrowth of follicles before weaning a third experiment started altrenogest treatment 3 d before weaning. This did not suppress follicle growth, but fertility was improved after altrenogest treatment in primiparous sows with compromised body condition at weaning. A fourth experiment attempted to stimulate follicle growth before weaning using split-weaning (reducing litter size to the 6 smallest piglets 3 d before complete weaning) and found that split-weaning resulted in lower embryonic survival, especially in sows with large follicles at weaning or high follicle growth during treatment. Because in all experiments sows showed follicle growth during altrenogest treatment, it was suspected that LH release was not completely suppressed during altrenogest treatment. Therefore, a fifth experiment investigated LH pulsatility during the last day of altrenogest treatment and indeed showed that LH release was suppressed during only a part of the 24 h between altrenogest administrations. Finally a last experiment showed a release pattern of both FSH and estradiol that varied over the day related with the moment of daily altrenogest administration. Further, a decrease of estrogenic activity was found during the second week of post weaning altrenogest treatment, probably as a result of reduced LH responsiveness. The level of estrogenic activity was related to weight loss during lactation. Therefore, it is assumed that LH and FSH release during altrenogest treatment stimulate follicle growth, but that levels are not high enough to sustain outgrowth of the follicles to pre-ovulatory sizes and, as a result, follicles go into atresia after on average 5-8 d of treatment. This may explain why long altrenogest treatments (12-15 d) result in improved fertility, short altrenogest treatments (3-4 d) have little effect on fertility and intermediate altrenogest treatments (5-8 d) may reduce fertility. As there is large variation between sows (parity, lactational burden, follicle size), this may also affect their response to altrenogest treatment. In general, to improve reproductive performance, it is recommended to start altrenogest treatment 3-6 h before weaning and to apply intermediate treatments (5-8 d) only in primiparous sows that suffered a severe lactational burden and are in low body condition at weaning.

Preface

More than 4 years ago I started this PhD, because I felt that there was still so much more to learn...and learning I did the past 4 years! Not only about reproductive physiology, but also about how to perform science, about how to manage experiments under difficult circumstances, about how to take decisions independently and bear the consequences, about how to teach students, about cultural differences and how this can lead to misunderstandings and about how one should appreciate the opportunity to get educated. Therefore, I am proud to present this thesis. I hope that it reflects the honor and pleasure I felt while writing it. Of course, this is not just my work; many people have been there to help and guide me, both professionally as personally and without them I would not stand where I stand today. Therefore to all of you; THANK YOU!

First of all I would like to thank my sponsors; Intervet Schering Plough Animal Health and Janssen Animal Health; thank you Marc, Hilduard and Jan for your trust and for giving me the possibility to extend my knowledge on reproductive physiology.

Second I want to thank my supervisors: Nicoline, thank you for your patience. I know that having me conducting the experiments on my own, so far away, was not easy to supervise, but I have enjoyed our cooperation a lot and you have taught me so much over the past 4 years. Thank you for always being there when I needed you (professionally and personally), for always finding the right track to follow and for making me to get organized; I will never forget to use a centimeter when measuring back fat anymore…Bas, thank you for having faith in me from the very beginning, even though my job interview was not exactly a success… Also thanks for always wearing your pink glasses when problems appeared, for your time and input in this thesis and for a memorable drive through the Argentinean country side where you showed me that traffic lights do exist in Argentina as well…

Of course there are many more people that I am very grateful to. Marc Antoine, thank you for your great suggestions and help with analyses and writing of the manuscripts. Bjorge, thank you so much for your help in the last experiment; without you I could not have done it. Rudie, also thanks you for all your help with the endocrine analyses of the last experiment. Nanette and Lora I am very grateful to the both of you for all your help with administrative and financial issues. Marcel, thank you for listening to me during all the therapeutic sessions between Raalte and Wageningen ☺. Wouter, Frits, Anne and Lia thank you for helping out with the blood sampling procedures. Also I would like to thank everyone from the experimental facilities "De Haar" for taking care of "my" sows, especially Ries, Ben, Sander, Rinie and of course Peter; thank you for the cappuccino's at the moments when I needed them most. Also thanks to drs. R. van Leeuwen for editing the first draft of this thesis.

For this thesis 5 experiments were conducted, which would have been impossible without the help of students. In order of appearance; Patricia, Gilda, Merel, Iris, Sander, Elian, Marlies and Martine; thank you for all the energy, hard work and long hours with the piggies. Anniek, thank you for your trust, your contribution to the Monte Buey trial and for all the good times we have had together.

Most of the experimental work of this thesis was done in Argentina, so I would like to express my gratitude in Spanish to the people who helped me out there.

Por este tesis recibí la ayuda de varias empresas y muchas personas a quienes me gustaría expresar mi agradecimiento. Primero, Dra. Sara Williams y la Universidad Nacional de la Plata por su ayuda. Sara, gracias por tu ayuda y por tu paciencia conmigo, fue un placer de trabajar con vos. Supermercados Alvear S.A. y empleados por permitirme de hacer el primer experimento en su establecimiento aunque no hablaba casi nada de castellano todavía y por ayudarme con el trabajo práctico. Dr. Mario Tome, gracias por tus consejos y tu ayuda con la faena de las cerdas. Universidad Nacional de La Pampa por permitirme el uso de sus instalacciones y materiales. Dra. Mirta Koncurat por su ayuda en el primer experimento. Dr. Julian Bartolome por su ayuda en los experimentos y el uso de su laboratorio. Julian, aprendí muchísimo de vos y estoy muy agradecida por todo. Te deseo todo lo mejor. Dr. Pablo Villafañe, gracias por tu ayuda conseguir una establecimiento para mi segundo experimento. Isowean S.A. me dio la posibilidad de hacer 2 experimentos usando sus instalaciones. Estoy entonces muy agradecida a Lisandro Culasso, Fernando Villavicencio, Dr. Diego Goñi por su participación y tambien a Dra. Lujan Mattio por su ayuda con el trabajo práctico. Gracias tambien a todos los empleados de Isowean quienes me ayudaron con el trabajo. Mis amigas Alejandra, Cristina, Silvana y Veronica, gracias por su apoyo.

And then of course, one cannot survive without the love and support of loved ones. Dear family and friends thank you for your understanding and patience with me when I was completely absorbed by my thesis…..

Mama, dit proefschrift is niet voor niets aan jou opgedragen; ik ben je enorm dankbaar voor alles wat je voor me doet en voor de buitenproportionele hoeveelheid doorzettingsvermogen die je mij hebt meegegeven. Dit wordt door anderen weleens verward met koppigheid maar is me de afgelopen jaren erg goed van pas gekomen. Papa, dankjewel voor je interesse in het onderzoek en voor je steun door dik en dun. Ik weet dat je zelf ook graag had willen promoveren en ben dan ook heel blij dat we dit moment samen zullen delen omdat je als paranimf aan mijn zijde zit. Lieve Ruben, Anniek en Sebastiaan dankjewel dat jullie er altijd voor me zijn, ik weet niet wat ik zonder jullie zou moeten en Anniek: de voorkant van dit proefschrift is een waar meesterwerkje geworden; dankjewel! Ineke, jij verdient ook een plaatsje in dit dankwoord, want zonder jouw ontelbare peptalks zou ik hier nu niet staan. Mijn paranimfen Lotte en Charissa, wat fijn dat jullie deze "duobaan" op je willen nemen en mij bij willen staan; ik ben heel blij met jullie vriendschap. Mijn "lotgenoten" Anne en Ana, dankjewel voor jullie gezelschap, steun en humor; zet m op met jullie eigen "laatste loodjes". Lieve Greetje, ik ben met je eens dat het tijd is dat ik nu eens wat ga bijdragen aan de maatschappij. Oftewel, HORA EST…….maar niet voordat ik nog een laatste persoon bedank…

Finalmente, porque siempre se guarda lo mejor para el último: Juan, sin vos no existía esta tesis. Vos me ayudaste en 4 de los 5 experimentos, siempre estás ahí cuando te necesito y fue tu fe en mí que me ayudó seguir adelante; te quiero mucho…

Chapter 1

General introduction

1.1 Introduction

Generally litter size increases with parity and largest litter sizes are found in parity 3-5 (Koketsu et al., 1999). However, in many sows the second litter is similar or even smaller than the first litter (Morrow et al., 1990). Suboptimal reproductive performance in the second parity is referred to as the second litter syndrome (Morrow et al., 1989) and is expressed as an increased weaning-to-estrus interval (Zak et al., 1997), a small litter size (Morrow et al., 1989) and a low farrowing rate. Decreased reproductive performance in the second litter may decrease sow longevity, because culling rates increase with decreasing reproductive performance (Sasaki and Koketsu, 2008; Hoving et al., 2011).

The cause of the second litter syndrome is attributed to consequences of a negative energy balance (NEB) during first lactation on follicle development, which may also affect embryo mortality during early pregnancy (Almeida et al., 2000; Algriany et al., 2004). The effects of NEB on weaning-to-estrus interval, nowadays are less pronounced since intervals to estrus have become less variable (Knox and Zas, 2001; Tummaruk et al., 2001; Belstra et al., 2004; Hoving et al., 2010).

During lactation follicle development is minimal because of low serum gonadotropin concentrations (for a review see: Schwarz et al., 2009), nevertheless a steady increase in average follicle size is seen. Only 2% of the follicles present grow to a size of 4 mm (Kunavongkrit et al., 1982) and follicles larger than 5 mm are rarely seen (Lucy et al., 2001). Follicle size is therefore small (3 mm) at weaning (Lucy et al., 2001). The stress of separation from the piglets at weaning, combined with the removal of the suckling stimulus, causes an increase in the level of FSH (Shaw and Foxcroft, 1985) and an increase in pulsatile LH release (Shaw and Foxcroft, 1985; Quesnel and Prunier, 1995). FSH stimulates growth of small (antral) follicles and stimulates the number of follicles in a cohort (Shaw and Foxcroft, 1985; Madej et al., 2005). Pulsatile LH release allows for selection and growth of the larger (antral) follicles (Driancourt et al., 1995) that eventually grow out to pre-ovulatory sizes. Morbeck et al. (1992) estimated that it takes a newly formed, antral follicle about 2 weeks to grow from 0.4 mm to 3 mm in size. This means that the antral follicle population present at weaning started to grow during the last 2 weeks of lactation. Development of the antral follicle pool during lactation can be impaired because of a low nutritional status of the sow (Quesnel et al., 2000). Zak et al. (1997[a]) demonstrated that feed restriction during lactation reduced LH release during lactation. So, a negative energy balance during lactation may change the endocrinological environment in which follicles mature, resulting at weaning in a follicle pool that matured under suboptimal conditions with reduced follicle quality and oocyte competence (Zak et al., 1997[b]). Restricted feeding may also directly affect follicle quality (reduced estrogenic activity) and reduce plasma IGF-1 levels which affects oocyte competence (Ferguson et al., 2003). Recruitment of these impaired follicles results in lower ovulation rates (Hazeleger et al., 2005; Zak et al., 1997[a]), impaired embryo development (Algriany et al., 2004; Zak et al., 1997[a]) and increased embryo mortality (Almeida et al., 2000; Zak et al., 1997[a]). This may subsequently reduce farrowing rates and litter sizes.

Because primiparous sows have limited feed intake capacities, their feed intake during lactation usually does not meet the requirements for milk production, resulting in substantial body

weight and back fat loss (Everts et al., 1994). A complicating factor in primiparous sows is that they are not full grown, so they require energy for growth. Hoving et al. (2010) found that a higher weight gain from first insemination to first weaning is associated with an increase in pregnancy rate and larger litter sizes after post weaning insemination. This makes primiparous sows especially sensitive for reduced reproductive performance in the subsequent litter, (e.g. Zak et al. 1997[a]; Thaker and Bilkei, 2005).

A logical approach to prevent the occurrence of the second litter syndrome is to stimulate nutritional intake during lactation in primiparous sows. However, the possibilities to do so are often limited. Chances are that with the current selection for litter size, the possibilities may even be more limited in the future.

Allowing the sow to recover from lactation by inseminating at second estrus after weaning (skip-a-heat) improves reproductive performance (Morrow et al., 1989; Clowes et al., 1994; Vesseur et al., 1997). This improved reproductive performance is largely attributed to higher embryo survival rates (Clowes et al., 1994). During the recovery period the metabolic status of the sow improves (Clowes et al., 1994) which results in improved levels of glucose, insulin and leptin. The improved status of metabolic hormones provides a signal to the HPO axis (Quesnel, 2009), resulting in resumption of normal GnRH release from the hypothalamus. This stimulates LH (pulsatile) and FSH release and allows for follicle development in more optimal conditions than during lactation, leading to improved follicle quality as can be seen in a higher progesterone production of the CL's after ovulation (Clowes et al., 1994). The improved metabolic status results in increased IGF-I levels, which directly stimulates improved follicle quality (enhanced estrogenic activity) and oocyte competence (Ferguson et al., 2003). The disadvantage of skip-a-heat is that the number of non-productive days per sow increases and that detection of second estrus is more difficult (Clowes et al., 1994). Providing a shorter recovery period after weaning by daily administration of altrenogest, a progesterone analogue, may also improve reproductive performance while not extending the recovery period too much.

The aim of this thesis was to develop a better understanding of the effects of altrenogest after weaning on sow physiology and follicle development and the relation with subsequent fertility. This should lead to strategies to improve reproductive performance in the second litter and extend the productive life of sows. Before the aim and outline of this thesis will be described in detail, the following paragraphs will first provide a historical overview of the use of altrenogest as a reproductive management strategy.

1.2 Post weaning altrenogest treatment

A strategy to overcome the effects of a negative energy balance during lactation is the use of a progestagen treatment after weaning. Postponing estrus after weaning, by daily oral administration of a progesterone analogue (altrenogest) allows for a recovery time after weaning, which has been found to positively affect subsequent ovulation rate (Koutsotheodoros et al., 1998; Patterson

et al., 2008), early embryonic development (Martinat-Botté et al., 1994), fetal development (Patterson et al., 2008), farrowing rates (Morrow et al., 1989; Martinat-Botté et al., 1994) and litter size (Morrow et al., 1989; Morrow et al., 1990; Martinat-Botté et al., 1994). It is yet unclear what causes this improved reproductive performance after Altrenogest treatment, but it is thought to be related to improved follicle development and follicle quality during treatment.

The orally active progestagen Altrenogest is a synthetic trienic C21 steroidal progestomimetic and member of the 19-nor-testosterone group (for the chemical structure see Figure 1). The most important effects of Altrenogest are progestomimetic and anti-gonadotrophic. It also has weak estrogenic, anabolic and androgenic effects, but has no corticoid or anti-inflammatory effects. Altrenogest's liposolubility allows it to penetrate the target cells of the hypothalamus easily, where it binds to specific receptors (Committee for Veterinary Medical Products, emea.europa). This causes a negative feedback on GnRH release from the hypothalamus resulting in inhibition of LH and FSH release from the anterior pituitary (Stevenson et al., 1985). Altrenogest is currently registered to synchronize estrus in gilts and in some countries (Belgium, UK) also to postpone estrus after weaning (Committee for Veterinary Medical Products, emea.europa).

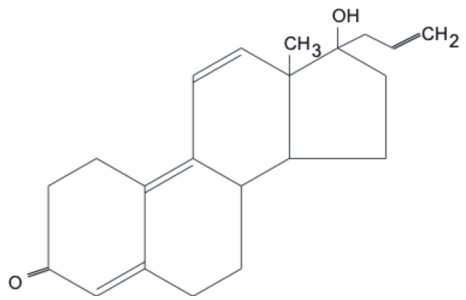

Figure 1. Chemical composition of altrenogest. Adapted from Flowers. 2002.

After oral administration of 20 mg of altrenogest peak levels are reached within 3 to 6 hours. Plasma concentrations decline biphasically, with an elimination half-life of about 10 days in pigs. The main routes for elimination are through bile via the faeces and urine. Data indicate that, in line with all steroids, the major metabolic pathway for altrenogest is oxidation and conjugation. The metabolites of Altrenogest show little or no hormonal activity (Committee for Veterinary Medical Products, emea.europa).

The first trials that used Altrenogest to control estrus and ovulation in pigs were performed more than thirty years ago. Davis et al. (1979) found effective synchronization of estrus and higher ovulation rates, but also a higher incidence of cystic follicles for gilts that were fed altrenogest (12.5 mg/day) for 18-19 days, compared to control gilts. Redmer and Day (1981) also found successful synchronization of estrus but an increase in the incidence of cystic follicles in gilts that received 2.5 mg/day compared to gilts that received 15 mg/day. In their study plasma E_2 levels were higher in sows that received 2.5 mg altrenogest than in sows that received 15.0 mg of altrenogest, but LH levels were low (<1.5 ng/ml) throughout altrenogest treatment. Estrus was effectively synchronized

5-7 days after removal of altrenogest in gilts given 15 mg of altrenogest, whereas in the 2.5 mg group an increased incidence of anestrus occurred, due to the development of cystic follicles.

So it seemed that a minimum dose was required to assure estrus after treatment. Kraeling et al. (1981) found that 10 mg/day was needed to effectively synchronize estrus in gilts. A longer duration of treatment (18 days) resulted in better synchronization of estrus of gilts, that initiated treatment at different stages of the estrous cycle, than a shorter (14 days) treatment period (Stevenson and Davis 1982). To establish optimal dosage in gilts, Varley (1983) compared reproductive performance of gilts that received 16 mg, 20 mg or no altrenogest for 18 days and found no differences in onset of estrus, but larger litter sizes in gilts that received 20 mg. Later studies also found a positive effect of the use of altrenogest on subsequent gilt reproduction (increased farrowing rate and litter size, Martinat-Botté et al., 1990; increased pregnancy rate and ovulation rate, Martinat-Botté et al., 1995).

Soon after the first experiments with gilts, altrenogest was also used in sows to improve reproductive performance of the subsequent cycle. Boland (1983) was one of the first to administer Altrenogest to sows to synchronize estrus. Primiparous sows were fed 20 mg of Altrenogest for either 3 or 7 days from weaning onwards or served as untreated controls. No estrus was observed during treatment and over 80% of the treated sows had shown estrus by day 8 after altrenogest withdrawal versus 56% of the control sows. This is in accordance with the findings of Martinat Botté et al., (1985) who also found improved estrus synchronization for altrenogest-treated sows. To establish the best timing of treatment Kirkwood (1986) conducted an experiment with primiparous sows where sows were treated for 7 days from weaning onwards. No differences in (the long) weaning to estrus interval (10.0 *vs.* 12.6 days for control and altrenogest, respectively) or pregnancy rates were found, but altrenogest treated sows had a numerical increase in live born piglets after treatment when compared with untreated controls (+1.5 piglets). He suggested that the larger litter size resulted from an increase in ovulation rate and that this was a direct consequence of the altrenogest treatment and not caused by an extended recovery period since the weaning to estrus interval was not extended.

After this, several studies have been conducted (for a complete summary, see Table 1): some with positive effects on reproductive performance (synchronization rate, Fernandez et al., 2005; ovulation rate, Koutsotheodoros et al. 1998; Patterson et al., 2008, embryo survival, Patterson et al., 2008) and others with no effect or a negative effect on reproductive performance (farrowing rate and litter size, Dos Santos et al., 2004; Werlang et al., *in press*).

Nowadays, it is hypothesized that the positive effect of altrenogest treatment may be attributed to the extended recovery period after weaning, which restores follicle development and thereby improves subsequent reproductive performance, as mentioned earlier in this chapter. Soede et al., (2007) studied follicle development during altrenogest treatment and indeed found larger follicle sizes at the final day of an altrenogest treatment when compared with follicle size at the late luteal phase in untreated, control gilts.

Why some studies find improved reproductive performance after post weaning altrenogest treatment while others find no effect or even a reduction in reproductive performance may be

Table 1. Reproductive performance after post weaning altrenogest treatment compared to untreated controls

Treatment							
Start treatment[1]	Lactation length	Parity	Dose (mg)	Length (days)	WEI	% synchrony of estrus	
D0	35	1	20	3	ns	ns	
D0	35	1	20	7	ns	ns	
D0	28	1	20	7	+9	-30	
D0	35	1	30	7	ns	+30	
D1	12	1	20	12	-0.9	+33	
D1	22	1	20	5	ns	+21	
D-2	18	2-7	15	7	+1.7	+10	
D-2	18	2-7	15	14	+2.2	-	
D0	21	1	20	5	-0.7	-10	
D0	21	1	20	5	-0.7	ns	

[1] Weaning = D0 [2] Viable number of embryos on day 25-28 of gestation [3] sows were weaned after 12 days of lactation [4] Live fetuses on day 50 of gestation [5] 2 different breeds were used in this study

attributed to e.g. different breeds, parity or lactational burden, but also to the diversity of application methods in those studies. Large differences are found in day of initiation of treatment (before or after weaning), duration of treatment and dosage of treatment (Table 1).

Studies vary in the moment of initiation of treatment. A study that started before weaning found improved reproductive performance (start 2 days before weaning, Patterson et al., 2008). Others started at the day of weaning and found improved reproductive performance (start at the day of weaning, Stevenson et al., 1985), no effect (start at weaning, Boland, 1983) or a negative effect on (start 3 hours after weaning, Werlang et al., *in press*) reproductive performance. Some started after weaning and found improved reproductive performance (start 1 day after weaning, Koutsotheodoros et al., 1998; Fernandez et al., 2005).

Also duration and dosage may affect the reproductive outcome. Daily administration for 3 to 7 days synchronizes estrus well in weaned sows (Boland, 1983; Martinat-Botté et al., 1985; Martinat-Botté et al., 1994), but a longer treatment is thought to result in better synchronization of estrus (Koutsotheodoros et al., 1998). Indeed Patterson et al. (2008) found better synchronization of estrus after 14 days of altrenogest than after 7 days. The minimal recommended dose in gilts was found to be 10 mg (Kraeling et al., 1981), but no such data are available for sows. Depending on the country of registration, altrenogest is fed at a daily dosage of 15 mg or 20 mg, which both have found to synchronize estrus effectively in sows. However, Kauffold et al. (2007) found a higher incidence of cystic follicles in sows treated with 16 mg compared to sows treated with 20 mg of altrenogest for 15 days after being diagnosed non-pregnant on days 21-35 after insemination.

Results					Reference
	Ovulation rate	Pregnancy rate	Farrowing rate	Litter size	
	-	ns	ns	ns	Boland, 1983
	-	ns	ns	ns	Boland, 1983
	-	-	+22%	ns	Stevenson et al., 1985
	-	ns	-	ns	Kirkwood et al., 1986
	+1.5	-	-	+2.6[2]	Koutsotheodoros et al., 1998[3]
	-	ns	-	ns	Fernandez et al., 2005
	+3.4	ns	-	ns	Patterson et al., 2008
	ns	-		+1.8[4]	Patterson et al., 2008
	-	-	-14%	-1.7	Werlang et al., *in press*[5]
	-	-	-30%	ns	Werlang et al., *in press*[5]

1.3 Aim and outline of this thesis

Since there is no established protocol for optimal post weaning altrenogest use (initiation, dose and duration of treatment) and the reproductive outcome of post weaning altrenogest treatments is variable, there is a need for a better understanding of the working mechanism of altrenogest and its effect on follicle development. The aim of this thesis was to develop a better understanding of the use of altrenogest after weaning as a strategy to overcome the negative effects of first lactation on follicle development and subsequent reproductive performance. For this purpose, follicle development and endocrine changes during and after post weaning altrenogest treatments (differing in dose and duration) were investigated and this was related to subsequent reproductive performance.

In Chapter 2 different post weaning altrenogest treatments were used to develop a better understanding of follicle growth during altrenogest treatment and the effect of dosages and duration of treatment on follicle growth and reproductive performance. Follicle development was studied daily from weaning till ovulation and sows were slaughtered 4-5 days after ovulation to study ovulation rate, CL quality and embryo development. The increase in follicle size, as seen during altrenogest treatment, was not affected by dose. So, in Chapter 3 altrenogest treatments of different durations, but at same dosage, were used to study follicle development and relate this to subsequent farrowing rate and litter size. Follicle size at initiation of treatment was found to affect reproductive outcome (farrowing rate). To investigate this further, 2 other experiments were conducted in which

an attempt was made to create more variability in follicle size at weaning. The first one (Chapter 4) attempted to prevent outgrowth of follicles before weaning, using different pre weaning altrenogest treatments. The second experiment (Chapter 5) used split-weaning (reducing litter size to six piglets during the last 3 days of lactation) in half of the (multiparous) sows, to stimulate follicle growth before (complete) weaning.

To understand the endocrine background of the increased follicle size during altrenogest treatment a last experiment was performed. Chapter 6 describes LH release during the last day of a 15 day altrenogest treatment and relates this to subsequent ovulation rate. In Chapter 7 pulsatile LH release around weaning and subsequent FSH release, follicle size and estradiol levels during a 15 day post weaning altrenogest treatment are described.

Finally, in the general discussion (Chapter 8) results of all experiments using altrenogest in weaned sows are combined and their reproductive results discussed based on the acquired knowledge. Further, scientific and practical implications for the use of altrenogest treatment are given.

Chapter 2

Post weaning altrenogest treatment in primiparous sows;

the effect of duration and dosage on follicular development and consequences for early pregnancy

J.J.J. van Leeuwen[1], S.I. Williams[2], B. Kemp[1] and N.M. Soede[1]

[1]Adaptation Physiology Group,
Department of Animal Sciences, the Netherlands
[2]Facultad de Ciencias Veterinarias,
Universidad Nacional de La Plata, Argentina

Animal Reproduction Science 119 (2010) 258-264

Abstract

Our objective was to investigate follicle development in sows during and after different altrenogest treatments post weaning and relate this to subsequent ovulation rate and embryonic development. Primiparous UPB sows (n = 47) were randomly assigned to (weaning = Day 0): control (no altrenogest, n = 12), RU8-15 (15 mg of altrenogest, Day -1 till Day 7, n = 12), RU8-20 (20 mg of altrenogest, Day -1 till Day 7, n = 12) or RU15-15 (15 mg of altrenogest, Day -1 till Day 14, n = 11). From weaning onwards, trans-abdominal ultrasound was performed daily. Sows were slaughtered on Day 4 or 5 after ovulation.

Follicle size increased during altrenogest treatment and reached a plateau around Day 6, regardless of dose (4.6 ± 1.5, 4.6 ± 1.6, and 4.6 ± 1.6 mm for RU8-15, RU8-20 and RU15-15, respectively). This increase resulted in larger follicles (P = 0.0002) at the onset of the follicular phase (i.e. time of weaning for control sows and 24 h after last administration of altrenogest for treated sows); 4.8 ± 1.8, 5.0 ± 1.3, 4.8 ± 1.1 and 3.0 ± 0.8 mm, for RU8-15, RU8-20, RU15-15 and controls, respectively. Pre-ovulatory follicle size tended (P = 0.07) to be larger for treated animals (7.9 ± 2.4, 7.9 ± 0.7, 8.6 ± 1.3 and 6.9 ± 0.9 mm for RU8-15, RU8-20, RU15-15 and controls, respectively). The interval follicular phase-estrus was shorter (P = 0.001) for treated animals. Treatment did not affect ovulation rate or early embryonic development. However, for treated animals, the increase in follicle size during treatment was positively related with ovulation rate (P = 0.05). In conclusion, post weaning treatment with altrenogest of first litter sows influenced follicle size and shortened the follicular phase, but did not affect ovulation rate or early embryonic development.

Keywords

Follicle development, Sows, Altrenogest, Ovulation rate, Embryonic development

2.1 Introduction

Many first litter sows suffer from a suboptimal reproductive performance in the second breeding cycle including an increased weaning-to-estrus interval, a low farrowing rate and a smaller litter size (Morrow et al., 1989). These negative effects on reproduction are associated with a negative energy balance during first lactation (e.g. Thaker and Bilkei, 2005). Several authors have shown that feeding levels either during lactation or during the luteal phase of the estrous cycle affect the antral follicle pool and subsequent follicle development (Quesnel et al., 2000), ovulation rate (Hazeleger et al., 2005) and also oocyte development (Zak et al., 1997[a]), contributing to embryo development (Algriany et al., 2004), leading to increased embryo mortality (Almeida et al., 2000).

Postponing mating of first litter sows to the second estrus after weaning ('skip-a-heat') consistently improves subsequent reproductive performance (Morgan Morrow et al., 1990; Clowes et al., 1994). Also postponing estrus for a shorter period, by administration of a progesterone analogue (altrenogest) from weaning onwards, positively affects subsequent reproductive performance (Morgan Morrow et al., 1990; Koutsotheodoros et al., 1998; Martinat-Botté et al., 1994; Martinat-Botté, 1995; Patterson et al., 2008). It is unclear what the cause is of this improved performance after altrenogest treatment, but it may be related to improved follicle development during this period. Until now follicle development during altrenogest treatments in post weaning sows has not been studied. It is our hypothesis that during altrenogest treatment, follicle development (measured as an increase in size of the largest follicles) improves and as a consequence, ovulation rate and early embryonic development also improve. If true these effects may be dependant on the dose and duration of altrenogest treatment. Testing this hypothesis will provide insight into post weaning follicle dynamics and its consequences for reproductive processes in sows and is needed to develop optimal altrenogest treatment schemes to improve second litter fertility.

Therefore, the current experiment used first litter sows to study follicle growth during and after altrenogest treatments differing in dosage and duration and relate this to subsequent ovulation rate and early embryonic development.

2.2 Materials and methods

2.2.1 Animals, housing and diets

A study was conducted during spring and summer from October 2007 to March 2008 in a commercial swine herd in Intendente Alvear, Argentina (35° 14' 0" S, 63° 35' 0" W) in accordance with the Argentine law for the protection of animal welfare (Ley penal 14346). Pregnant gilts (n = 47, UPB Genetic World) entered the maternity facilities on average 5 to 9 days before farrowing and were individually housed in farrowing crates. Postpartum cross-fostering was performed within 3 days after farrowing to adjust litter size to 9-11 piglets. During lactation sows received a

corn and soybean based diet, formulated to contain 14.07 MJ digestible energy/kg with 17% crude protein and 1% lysine, to a maximum of 8 kg per day. At weaning, at day 21 (18-24) of lactation, piglets were individually weighed and the sows moved to individual crates in another barn where they were fed twice a day (8 a.m. and 4 p.m.), to a maximum of 4 kg of the lactation ration.

Barns were equipped with individual drinking nipples providing ad libitum access to water for all sows. Daily minimum and maximum environmental temperatures were registered throughout the experiment. Average temperature varied between max. 23.2 ± 5 °C and min. 11.7 ± 2 °C (October) and max. 31.2 ± 4 °C and 17.3 ± 4 °C (December) (range of maximum temperature; 14.2 °C-38.1 °C). Whenever environmental temperature exceeded 25 °C spray coolers were activated in the gestation barn.

Sows were weighed and P2-back fat was measured using A-mode ultrasound (lean-meater®, Renco Corporation, Minneapolis, USA) (6.5 cm off the midline at the level of the last rib) at entering of the farrowing facilities. Sow weight before farrowing was corrected for the weight of the litter at birth, estimated placenta and amnion weight (216 g/piglet) and estimated fetal growth during the days between weighing of the sow and farrowing (60 g/fetus/day; based on Leenhouwers et al. (2002) and van Rens et al. (2002; 2005). Sow weight and P2 back fat were also assessed at weaning and at slaughter.

2.2.2 Treatments

The day before weaning (weaning = Day 0), sows were randomly assigned to one of four treatments; control (no altrenogest treatment; n = 12), RU8-15 (15 mg altrenogest, Day -1 till Day 7; n = 12); RU8-20 (20 mg altrenogest, Day -1 till Day 7; n = 12) and RU15-15 (15 mg altrenogest, Day -1 till Day 14; n = 11). These dosages were chosen because these represent the commercially used dosages for extension of the luteal phase in pigs (15 mg in North America and 20 mg in Europe and South America). Altrenogest (Regumate®, Intervet Productions S.A.), a progesterone analogue containing 4 mg of altrenogest/mL, was administered daily, as a top dressing, over a small portion of the morning feed to assure full ingestion of the entire dose, using a 5 mL syringe.

2.2.3 Estrus detection and insemination

From weaning onwards, estrus detection was performed at 8.30 a.m. and 4.30 p.m. by a trained farm technician using fenceline boar contact and a back pressure test. Sows were considered to be in estrus when they exhibited a standing reflex in the presence of the boar. Onset and end of estrus were recorded for each sow. Sows were inseminated on the first insemination session after first detected estrus and inseminations were repeated twice daily to a maximum of 4 inseminations as long as the sows exhibited a good standing reflex. Inseminations consisted of an 80 mL dose of semen containing approximately 4.4 x 10^9 spermatozoa derived from the farm's Large White boars. Semen was diluted with a semen extender (BTS® extender, Mini Tüb, Germany) and stored for a maximum of 3 days after collection.

2.2.4 Follicle development

From weaning until day of ovulation, trans-abdominal ultrasonography (Piemedical Aquila, Maastricht, the Netherlands) was performed daily with a 5 MHz multi convex transducer. One ovary was scanned each time since it was considered to be representative for the contra lateral one (N.M. Soede; unpublished results). Recorded images were subsequently reviewed using the cineloop application of the scanner. The diameters of the five largest follicles were measured and averaged. Ovulation was presumed to have occurred when previously present pre-ovulatory sized follicles (6-9 mm) had disappeared. This was confirmed by an additional scanning the next day. Since follicle size can decline during the last hours before ovulation (Soede et al., 1998), pre-ovulatory follicle size was defined as the largest average follicle size during estrus.

2.2.5 Ovulation rate and embryo development

Inseminated sows were slaughtered by stunning and exsanguination at 96 h (n = 8) or 120 h (n = 36) after ovulation to recover their reproductive tracts. The number of corpora lutea was assessed on both ovaries and size of all corpora lutea and total luteal weight were assessed. Furthermore, when present, cystic follicles (>11 mm) were counted on both ovaries.

Embryos were recovered and processed as described previously (Soede et al., 1995) to determine embryo quality, morphology, numbers of nuclei and sperm cells in the zona pellucida. Fertilization rate was established by dividing the number of embryos with at least 2 nuclei by the total number of recovered embryos and oocytes. The percentage of good embryos was established by dividing the number of good embryos as judged after flushing by the total number of fertilized oocytes. Per sow the average number of sperm cells in the zona pellucida, average and SD of the number of nuclei and average and SD of the number of cell cycles (^{2}log of the nuclei count) were assessed. Embryo development was evaluated in more detail by counting the percentage of embryos that developed to compacted morula (CM) stage and further (well developed embryos). As a measure for the within-litter variation in the development of viable embryos, the range in number of cell cycles was assessed for the embryos in a litter that had more than 90% of the litter average in cell cycles, since we assumed that the 10% less developed, retarded embryos would die at an early stage of pregnancy.

2.2.6 Statistical analysis

Data were analysed using SAS (SAS User's Guide: Statistics (Version 5) (1990) SAS Inst. Inc Cary, NC). Only sows with ovulation within 8 days after either weaning (Control sows) or end of altrenogest treatment (RU sows) were included. The onset of the follicular phase (Day 0) was defined to start at the time of weaning for control animals. For treated animals the follicular phase does not start at the moment of last daily altrenogest administration, since altrenogest treatment suppresses follicle development and prohibits estrus when administered at 24 hour intervals. Previous studies have found longer intervals from last altrenogest treatment to estrus compared with weaning-to-estrus intervals of control animals (Patterson et al., 2008; Dos Santos et al., 2004). Therefore, the arbitrary choice was made to define the onset of the follicular phase in treated animals to be 24h after last altrenogest administration.

Normality of parameters was checked with the Univariate procedure of SAS; the normally distributed parameters (follicle size at different days, increase in follicle size during treatment, onset and duration of estrus, ovulation rate and embryo- and ovarian parameters) were analyzed with the GLM procedure of SAS with treatment as main effect. For embryo- and ovarian parameters (e.g. number of cell cycles, SD of cell cycles and average CL size) day of slaughter (4 or 5 days after ovulation) was always included as covariate.

For each of the parameters relevant co-variates were analyzed, e.g. for follicle size at weaning, effects of lactational parameters (weight of sow before farrowing and at weaning, litter size, weight loss and back fat loss) were considered and also the interaction with treatment. If the interaction was not significant, it was omitted from the model. To analyze the increase in follicle size during altrenogest treatment, the increase in follicle size between 2 consecutive days was compared using a Student's t-test for paired observations. For animals in the RU15-15 treatment, follicle growth during the first and last week of treatment was also compared with the student's t-test for paired observations.

Embryo quality (good embryos in the litter, expressed as a percentage of the recovered embryos) and embryo development (stage 1 (up to CM) vs. stage 2 (CM and further)) were analyzed for all animals slaughtered at Day 5 using the GENMOD procedure of SAS with treatment as factor in the model and sow as a repeated subject.

Relations between parameters were tested by calculating the Pearson correlation coefficients. Also the effect of average maximum temperature during lactation on lactational parameters and the effect of average maximum temperature during the follicular phase on subsequent reproductive performance were tested calculating the Pearson correlation coefficients.

A probability level of less than 0.05 was considered significant and a probability between 0.05 and 0.1 indicated that a difference approached significance (tendency). Whenever main effects were significant means were compared by the least significant difference method adjusted for multiple comparisons according to Bonferroni. Data are presented as means ± standard deviation unless stated otherwise.

2.3 Results

2.3.1 Animals

On average 9.3 ± 1.2 piglets were weaned after a 21 ± 3 day lactation, during which sows lost 8.0 ± 10.2 kg (4.3 ± 5.5%) of their body weight and 1.9 ± 2.2 mm of P2 back fat. No difference between treatments was found for any lactational parameter.

Of the 47 sows, 3 sows did not ovulate and were deleted from further analyses. One of them was a control sow with 4.3 mm sized follicles at weaning that grew to 6.9 mm at Day 6 and subsequently became cystic. She did not show estrus. The second sow was in treatment RU8-15 with 2.2 mm follicles at weaning. During treatment, follicles grew to 8.7 mm and thereafter became cystic (to 21 mm within 6 days) and she did not ovulate. The third sow was also in RU8-15, with follicles of 1.9 mm at weaning. During treatment, follicles grew to a size of 2.4 mm and subsequent follicle size never exceeded 5.7 mm and she did not show estrus.

2.3.2 Follicle development

Of the 44 remaining sows follicle size at weaning (Day 0) varied considerably between sows from 1.0 to 6.3 mm, but average follicle size at weaning (2.7 ± 1.1 mm) was not different between treatments ($P > 0.10$; Figure 1).

In the altrenogest treated animals follicle size increased during the first 24 h after weaning ($P = 0.002$) and then progressively increased during the next 5 days with a plateau reached at the 6th day after weaning of on average 4.6 mm (Figure 1).

The gradual growth during the treatment period resulted in a significant difference in follicle size at the start of the follicular phase (Day 0) between the treated animals and control animals (2.9 ± 0.8, 4.8 ± 1.8, 4.8 ± 1.4 and 4.9 ± 0.9 mm for controls, RU8-15, RU8-20 and RU15-15 respectively; Figure 1). This difference remained significant until Day 4 of the follicular phase, but thereafter follicle sizes were similar in all treatment groups. Pre-ovulatory follicle size tended ($P = 0.07$) to be larger for treated animals than for controls (6.9 ± 0.9, 7.9 ± 2.4, 7.9 ± 0.7 and 8.6 ± 1.3 mm for controls, RU8-15, RU8-20 and RU15-15 respectively).

2.3.3 Estrus, ovulation rate, embryo and luteal development

RU8-15 and RU8-20 had a shorter interval between start of follicular phase and onset of estrus than control animals ($P = 0.005$; Table 1). Duration of estrus was not affected by treatment, neither was ovulation rate or luteal weight (Table 1). Two animals had multiple cystic follicles; one sow in the RU8-15 group had 12 CLs and 5 cystic follicles at slaughter and one RU8-20 sow had 30 CLs and 6 cystic follicles at slaughter. A further 5 animals had 1 cystic follicle at slaughter; 1 in RU8-15, 2 in RU8-20 and 2 in RU15-15.

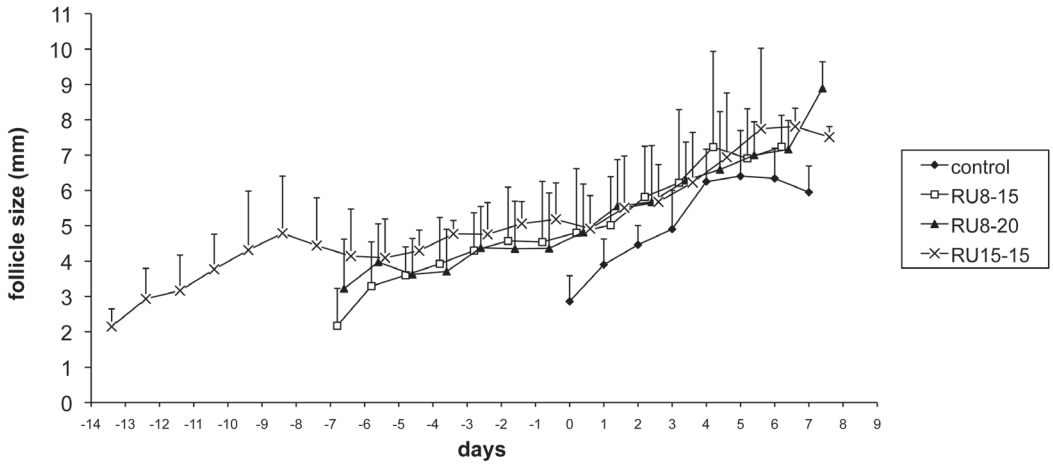

Figure 1. Follicle development (mean ± SD) during altrenogest treatment and the follicular phase. Day 0 = weaning for control sows and day after last altrenogest administration for RU8-15, RU8-20 and RU15-15.

Table 1. Estrus, ovarian and embryo parameters for control, RU8-15, RU8-20 and RU15-15 [ab] (mean ± SD)

	Control (n = 11)	RU8-15 (n = 9-10)	RU8-20 (n = 11-12)	RU15-15 (n = 10-11)
Estrus				
Onset estrus (d)[c]	6.5 ± 1.4[1]	4.6 ± 1.4[2]	4.7 ± 0.9[2]	5.2 ± 1.6[3]
Duration estrus (h)	52.4 ± 8.1	56.4 ± 18.8	54.0 ± 9.6	53.5 ± 9.8
Ovaries				
Ovulation rate	19.5 ± 3.1	18.9 ± 4.0	21.2 ± 5.4	18.8 ± 4.0
Average CL size (mm)	9.7 ± 1.5	9.5 ± 1.6	10.0 ± 2.2	10.3 ± 1.6
Total luteal weight (g)	7.58 ± 3.14	7.46 ± 3.33	9.81 ± 4.47	7.67 ± 3.40
P4 (ng/mL)	25.0 ± 2.0	24.5 ± 2.2	26.3 ± 1.9	27.4 ± 2.0
Embryos				
Unfertilized oocytes %	7.7 ± 13.7	10.7 ± 31.5	13.0 ± 28.3	6.2 ± 12.9
Good embryos %	66.3 ± 16.5	63.4 ± 29.5	63.4 ± 40.9	64.6 ± 28.8
Well-developed embryo's % (morula and up)[d]	84 ± 16	91 ± 9[1]	75 ± 35[3]	82 ± 20
Nuclei (mean number)[e]	59.3 ± 38.6	68.1 ± 26.1	62.0 ± 36.8	66.4 ± 47.7
Cell cycles (mean number)[e]	5.4 ± 1.2	5.8 ± 0.6	5.4 ± 0.8	5.3 ± 1.8
Cell cycles (SD)[e]	0.7 ± 0.3	0.8 ± 0.4	1.0 ± 0.8	0.6 ± 0.3
Cell cycle range of viable embryo's[e]	1.15 ± 0.16	1.23 ± 0.15	0.98 ± 0.22	1.22 ± 0.15
Sperm cells (median)	11	24	46	14

[a] P4, Nuclei, cell cycles and cell cycle range of viable embryos were corrected for embryo age (day of slaughter).

[b] 1 sow in control was not included because she did not show estrus, 2 sows in RU8-15 were not included because they did not show estrus, 1 sow in RU 8-20 and 1 sow in RU15-15 were deleted for embryo data, because at slaughter they had endometritis and embryos could not be examined well.

[c] Controls: from weaning; Treated animals: from 24h after last Altrenogest treatment

[d] Analysed based upon real number of embryos with logistic regression but presented as %

[e] Analysis done with all embryos classified as good

[12] $P = 0.005$

[13] $P < 0.1$

In total 643 embryos were recovered from 44 sows, which were analysed to assess quality and development (Table 1). Average fertilization rate of the recovered embryos and oocytes was 90.6%, which was not affected by treatment. The majority of the recovered embryos consisted of compact morulas (26%) and blastocysts (53%). No treatment effect was found on embryo quality (visual appraisal), number of nuclei in the embryos, number of accessory sperm cells, number of cell cycles or homogeneity of the litter (expressed as the range in number of cell cycles for embryos with more than 90% of the litter average in cell cycles) (Table 1). None of these parameters were related with follicle size at weaning, follicle size at the start of the follicular phase or pre-ovulatory follicle size.

2.3.4 Relations

Follicle size at weaning was negatively related to average weaning weight of the piglets (r = -0.36, $P = 0.02$) and positively related to weight of the sow at weaning (r = 0.37, $P = 0.02$).

For treated animals, the increase in follicle size during treatment, was related to follicle size at weaning (r = -0.54, $P = 0.0015$) and with ovulation rate (b=1.11; thus every 1mm increase in follicle size was accompanied by a 1.11 increase in ovulation rate $P = 0.05$).

Also for treated animals, follicle size at the end of treatment was positively related to weight of the sow at weaning (r = 0.50, $P = 0.003$) and tended to be negatively related to the total weight of the weaned litter (r = -0.29, $P = 0.1$).

Pre-ovulatory follicle size was found to be related to follicle size at the start of the follicular phase (r = 0.45, $P = 0.006$).

Average maximum temperature during the follicular phase which varied between 14.2 and 38.1 °C, tended to be related to average number of nuclei in the embryos (r = 0.29, $P = 0.07$) and average number of cell cycles of the embryos (r = 0.28, $P = 0.08$). Average maximum temperatures during lactation or the follicular phase were not related with any other parameters.

2.4 Discussion

The current study used primiparous sows to evaluate restoration of follicle development during post weaning altrenogest treatment. Altrenogest acts through a negative feed back mechanism on the hypothalamus, causing it to produce less GnRH which prevents the typical post weaning high frequency/low amplitude pituitary LH release which is responsible for outgrowth of follicles to ovulatory sizes (Kemp et al., 1998). During altrenogest treatment average follicle size remained less than 5 mm.

It was expected that the different dosages (15 versus 20 mg) of altrenogest used in the current experiment would lead to a different follicle size at the end of treatment. In this study however, follicle size at the end of a 8 day treatment was similar for sows treated with either 15 mg or 20 mg altrenogest daily (4.8 ± 1.8 *vs*. 4.8 ± 1.4 mm). A longer duration of the 15 mg treatment (for 14 days) did not increase follicle size at the end of treatment.

Follicle growth during treatment varied highly between individual animals. Some sows

showed a steady growth, while follicular growth in others showed a more "spiky" pattern and also in some sows a large decline in follicle size was seen, followed by an increase in follicle size during several days. This may indicate that a new cohort of follicles started to grow. Lucy et al. (2001) described follicle turnover in sows during lactation. They observed synchronized waves of follicular growth in lactating sows. Each wave consisted of a cohort of 20-30 follicles that grew from 2 mm to 4-6 mm after which the follicles regressed and a new cohort started to develop. Our observations seem to indicate that follicle turnover or follicular waves may not only occur during lactation, but also during altrenogest treatment after weaning. This turnover occurred only in few animals. We were therefore unable to calculate if there is a relation with subsequent reproductive performance.

Related to an increased follicle size at the onset of the follicular phase, treated animals also tended to have larger pre-ovulatory follicles than control animals. Soede et al. (1998) found that pre-ovulatory follicle volume was positively related to luteal weight at Day 5 after ovulation which may be relevant for later pregnancy. Similarly, a positive correlation between pre-ovulatory follicle size and subsequent luteal size has been observed for cows (Perry et al., 2005) and ewes (Murdoch and Van Kirk, 1998). However, in our study no relation between follicle- and CL-size was found.

The question remains as to whether a larger follicle is also a qualitatively better follicle, holding a better quality oocyte. Hunter and Wiesak (1990) found a correlation between follicular fluid volume and stage of oocyte maturation for non treated animals and a correlation between follicular diameter and stage of oocyte maturation for animals treated with PMSG followed 72 h later by hCG. In cattle, the relation between follicle size and oocyte competence has been well investigated. In experiments with oocytes derived from slaughterhouse material subjected to IVM, IVF and IVC, it was shown that embryos developed from oocytes, derived from larger follicles, were further developed than embryos developed from oocytes, derived from smaller follicles (Lonergan et al., 1994; Salamone et al., 1999). Extrapolating from these results it can be suggested that larger follicles are functionally better or at least contain more competent oocytes, so the larger follicles seen in treated animals in this experiment could result in improved subsequent fertility.

Despite larger follicles for treated animals, both at the onset of the follicular phase and at ovulation, no effect of treatment was found on ovulation rate or embryonic development. That no difference between the different altrenogest treatments was found may be explained by the fact that in all treatments, follicle growth was similarly restored within 7 days of treatment. Nevertheless, differences between the treatment groups and the control group were expected since the altrenogest treated animals had more time to recover from lactational weight loss. Several authors have found reduced post weaning reproductive performance when lactational weight losses exceeded 10 to 15% of body weight (Zak et al., 1997[b]; Thaker and Bilkei, 2005; Clowes et al., 1994). Similarly, Bracken et al. (2003) found smaller follicles 3 days post weaning for sows with low body condition scores and Clowes et al. (2003[b]) found that sows with high body mass had significantly more large follicles, higher follicular estradiol concentrations and higher

uterine weight at weaning than low body mass sows. So, it was expected that the effect of improved nutritional status after weaning during pre-ovulatory follicle development in altrenogest treated animals would improve reproductive performance since an increase in recovery time improves reproductive performance (Morgan Morrow et al., 1990; Clowes et al., 1994; Vesseur et al., 1997) presumably due to improved follicle and oocyte quality and subsequent embryo survival (Zak et al., 1997ab; Cosgrove, 1998). The sows in the current experiment on average only lost 8 kg of body weight during lactation, corresponding to only 4% of their body weight. Their lactational burden was therefore relatively low and may not have been sufficient to depress follicle growth to such an extent that it compromised follicle quality or subsequent ovulation rate.

This experiment was conducted during summer months and the relatively high temperature may have affected reproductive performance since high temperature is known to reduce appetite and body reserve mobilization (Prunier et al., 1997). An effect of temperature during lactation on lactational weight loss or back fat loss was therefore expected, but no such effect was seen in this study. The only tendency of a temperature effect in this study was a positive relation of the average (maximum) temperature during the follicular phase (14.2 - 38.1 °C) with average number of nuclei in the embryos and average number of cell cycles. This tendency for a positive effect on embryo development goes against the general belief that high temperatures may lead to increased early embryonic death (Christianson, 1992).

Despite the fact that the major cause of seasonal variation in reproductive performance in warm climates is related with the consequences of high temperature (e.g. Prunier et al., 1997) and in our study no relations with temperature were found, it is still possible that season may have negatively affected reproductive parameters in our study through changes in melatonin production associated with day length changes (e.g. Tast et al., 2001 and Ramirez et al., 2009). If this was the case, this may have caused a damping effect on the expected positive effects of the altrenogest treatment.

Nevertheless in our study a larger increase in follicle size during treatment was related with a higher ovulation rate, suggesting that sows with more compromised follicle development at the onset of altrenogest treatment would benefit more in terms of follicle development and subsequent fertility.

In this study, sows were slaughtered on Day 5 after ovulation, which allows for assessment of all oocytes/embryos, including assessment of fertilization rate and early degeneration. At this stage, no differences in embryo development were found between treatments. It is possible that treatment effects on embryo development or embryo survival are not expressed until a later stage of pregnancy.

In total 9 sows either developed complete cystic ovaries (n = 2), or did ovulate but had one (n = 5) or multiple (n = 2) persistent follicles that turned cystic. This was the case for 1, 0 and 1 of the 12 control sows (17%) and 1, 2, and 4 of the 35 altrenogest treated sows (20%). Sub-minimal dosage (2.5 mg/day) of altrenogest has been associated with high incidence of cystic follicles (Redmer and Day, 1981). The development of cystic follicles did not increase with daily dosages of altrenogest of 15 or 20 mg.

In conclusion, follicle size increased during the first 6 days of post weaning altrenogest treatment, independent of dosage, resulting in larger follicles for treated animals during the first four days of the follicular phase and also at ovulation. The follicular phase was significantly shorter in treated animals and the increase in follicle size during treatment was related with higher ovulation rates. However, in contrast to expectations, treatment with altrenogest did not improve average ovulation rate or embryo development, not even during a treatment period of 14 days. Therefore, in these sows, the extra open days due to the post weaning altrenogest treatment would probably not be compensated by improved reproductive performance.

More research is needed to investigate effects of duration and dose of altrenogest treatment on subsequent reproductive performance in animals with a stronger negative energy balance after lactation.

2.5 Acknowledgements

The authors thank Intervet International BV and Janssen Animal Health for their financial support, Supermercados Alvear S.A and its employees for their assistance in this study, dr. Mario Tome and his employees for assistance in the slaughter procedures, Universidad Nacional de La Pampa for the use of their facilities, dr. Mirta Koncurat for her cooperation, the students Patricia, Gilda and Gustavo for their participation, Intervet Brasil for supplying the Regumate and dr. Julián Bartolomé for providing the lab facilities.

Chapter 3

The effect of different post weaning altrenogest treatments of primiparous sows on follicular development, pregnancy rates and litter sizes

J.J.J. van Leeuwen*, S.I. Williams#, M.R.T.M. Martens§, J. Jourquin‡, M.A. Driancourt†, B. Kemp* and N.M. Soede*,

*Adaptation Physiology Group, Department of Animal Sciences, Wageningen University, the Netherlands
Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, Argentina
§ Intervet Schering Plough Animal Health, the Netherlands
‡ Janssen Animal Health, Belgium
† Intervet Schering Plough Animal Health, France

Journal of Animal Science 89 (2011) 397-403

Abstract

This study investigated follicular development during and after post weaning altrenogest treatment of primiparous sows in relation to subsequent reproductive performance. Primiparous sows (n = 259) were randomly assigned at weaning (D0) to 1 of 4 groups: control (no altrenogest, n = 71), RU4 (20 mg of altrenogest D-1 till D2, n = 62), RU8 (20 mg of altrenogest D-1 till D6, n = 65) or RU15 (20 mg of altrenogest Day-1 till D13, n = 61). Average follicle size (measured by ultrasound) increased during altrenogest treatment and resulted in larger follicles at the start of the follicular phase for RU4, RU8 and RU15 compared with controls (5.3 ± 0.9, 5.5 ± 1.3, 5.1 ± 1.2 and 3.4 ± 0.6 mm $P < 0.0001$, respectively). Farrowing rate was greater in RU15 (95%) than in RU8 (76%; $P = 0.04$). The RU15 group also had more piglets (2 to 3 more piglets total born and born alive; $P < 0.05$) than the other treatment groups. Follicular development at weaning clearly affected reproductive performance. At weaning average follicle size: small (< 3.5 mm), medium (3.5 to 4.5 mm) or large (≥ 4.5 mm), was associated with farrowing rates of 86%, 78% and 48%, respectively ($P < 0.001$). Sows with large follicles at weaning had low (71%) in RU4, very low farrowing rates (22%) in RU8, but normal farrowing rates in RU15 (83%). In conclusion, this study showed that 15 d of post weaning altrenogest treatment of primiparous sows may allow follicle turnover in sows that had large follicles at weaning and that this was associated with an improved reproductive performance. It also showed that shorter treatment with altrenogest (4 or 8 d) is beneficial for sows with small follicles at weaning, but is not recommendable for sows with large follicles at weaning.

Key words: altrenogest, follicle development, litter size, pregnancy rate, sow

3.1 Introduction

A negative energy balance during first lactation affects subsequent follicular development (Quesnel et al., 2000), ovulation rate (Hazeleger et al., 2005), oocyte development (Zak et al., 1997a) and embryo development (Algriany et al., 2004), and ultimately leads to embryo mortality (Almeida et al., 2000). As a result, primiparous sows commonly have increased intervals from weaning to estrus, reduced farrowing rates and reduced litter sizes (Morrow et al., 1989).

Administering an orally active progestagen from weaning onwards improves reproductive performance post weaning. Several studies have found greater ovulation rates (Koutsotheodoros et al., 1998), better synchronization of estrus (Martinat-Botté et al., 1994; Martinat-Botté, 1995) and improved embryo survival (Patterson et al., 2008) for primiparous sows treated with altrenogest post weaning. These positive effects are probably related to an improvement in follicular development. In a previous study, altrenogest-treated, primiparous sows were found to have larger follicles at the start of the follicular phase than non-treated controls, but these sows showed no improvement in embryo development at d 5 of pregnancy (van Leeuwen et al., 2010).

The current study was designed to investigate the relation between follicular development during and after post weaning altrenogest treatment and relate this to subsequent pregnancy rate, farrowing rate and litter size. We hypothesized that treatment with altrenogest would result in larger pre-ovulatory follicles and would positively affect pregnancy rate on d 30, farrowing rate and subsequent litter size.

3.2 Materials and methods

The study was conducted during spring, from October 2008 to December 2008 in a commercial swine herd in Monte Buey, Argentina (32°25'00"S, 62°27'00"W) following the Argentine animal welfare law (Ley penal 14346).

3.2.1 Animals, housing and diets

Pregnant gilts (n = 259; PIC23) entered the maternity facilities with individual farrowing crates on average 5 to 8 d before farrowing at 309 ± 15 d of age, when they weighed on average 243 ± 19 kg. On d 114 of gestation all sows received 10 mg intramuscularly **(i.m.)** of a $PGF_{2\alpha}$ analogue: dinaprost tromethamine (Lutalyse Pfizer Inc, NY USA). Post-partum crossfostering was performed within 3 d after farrowing to adjust litter size to 11 to 13 piglets. During lactation sows were fed a corn and soybean based diet formulated to contain 14.48 MJ DE/kg with 18 % crude protein and 1% lysine to appetite to a maximum of 8 kg/d. On day 20 (18 to 22) of lactation piglets were weaned (11 ± 2 piglets per sow) and sows were moved to individual crates in another barn and fed twice each day (0800 and 1700h) to a maximum of 4 kg/d of a corn and soybean based diet that was formulated to contain 13.65 MJ DE/kg with 14% crude protein and 0.7% lysine. All crates were equipped with individual drinking nipples providing water for all sows ad libitum.

Daily minimum and maximum environmental temperatures were registered throughout the experiment. Average temperature varied between a maximum of 28.0 ± 1°C and a minimum of 12.9 ± 3°C (October) and a maximum of 31.2 ± 5°C and minimum of 15.5 ± 4°C (November). When the environmental temperature exceeded 28°C, spray coolers were activated in the gestation barn.

Sows were weighed and backfat depth was measured using A-mode ultrasound (Lean-Meater, Renco Corporation, Minneapolis, USA) at the P2 spot (6.5 cm from the midline at the level of last rib) when entering the farrowing barns, at weaning and for altrenogest-treated sows also on the day after last altrenogest treatment. Farrowing weight was corrected for weight of the litter at birth + estimated placenta and amnion weight (216 g/fetus), and for estimated fetal growth during the time between weighing and farrowing (60 g/fetus/d) based on Leenhouwers et al. (2002) and van Rens et al. (2002, 2005).

Farrowing weight averaged 223 ± 18 kg and backfat depth 19.4 ± 3.7 mm. During lactation, sows lost 19.4 ± 16 kg (equivalent to 8.4 ± 7%) of their body weight, and 3 ± 3 mm of their backfat thickness.

3.2.2 Treatments

The day before weaning (weaning = D0) sows were randomly assigned to 1 of 4 treatments: control (no altrenogest treatment; n = 71), **RU4 (**20 mg of altrenogest D-1 to D2; n = 62), **RU8** (20 mg of altrenogest D-1 to D6; n = 65) and **RU15** (20 mg of altrenogest, D-1 to D13; n = 61). Five milliliters of altrenogest (Regumate, Intervet, http://www.intervetusa.com), a progesterone analog containing 4 mg of altrenogest/mL, was administered daily as a top dressing over a small portion of the morning feed to ensure ingestion of the entire dose.

3.2.3 Estrus detection and insemination

Starting at weaning, estrus detection was performed twice each day at 0830 and 1730 h with fence-line boar contact. Estrus was considered to start when a sow first exhibited a standing reflex during a back pressure test in the presence of the boar and to end when a sow first failed to exhibit a standing reflex. Sows were inseminated in the morning or afternoon after first detected estrus (0900 or 1700 h, respectively), and inseminations were repeated every am / pm for a maximum of 4 inseminations as long as the sow exhibited a standing reflex. Inseminations consisted of a 100 mL dose of semen that was obtained from the farm's boars (PIC 415), with a dosage of 4 x 10^9 sperm cells. The extender used was Merck III (Mini Tüb, Tiefenbach, Germany) was used. Semen was stored at 17°C for a maximum of 3 d.

3.2.4 Follicular development

Trans-abdominal ultrasound with a 5-MHz multi convex transducer (Aquila, Piemedical, The Netherlands) was performed to assess follicular development on the day of weaning, after 8 d of treatment for RU15, 1 d after last altrenogest treatment for treated sows, and on d4 of the follicular phase (4 d after weaning for controls and 5 d after last altrenogest treatment for treated

sows). The follicular phase was assumed to start at weaning for control animals and 1 d after last altrenogest administration for treated animals. One ovary was scanned each time because it was considered to be representative of the contra lateral ovary (N.M. Soede; unpublished results). Recorded images were subsequently reviewed using the scanner's cineloop application. The diameter of the five largest follicles was measured. Due to practical reasons, only 206 from the 259 animals (80%) were scanned.

3.2.5 Pregnancy rate and litter size

Inseminated sows were checked for pregnancy on d 30 after insemination with A-mode ultrasound (Scanopreg, Scanco, Ithaca, NY). On d 114 of gestation, pregnant sows received 10 mg i.m. of Lutalyse (Pfizer). At farrowing of the second litter, piglets were weighed and the number of total born piglets, the number of piglets born alive, the number of piglets born dead and the number of mummified fetuses were recorded.

3.2.6 Statistical analyses

Data were analyzed using SAS (SAS Inst. Inc., Cary, NC). Only sows showing estrus within 10 d after weaning (control sows) or 11 d after last altrenogest treatment (treated sows) were included (n = 235). Follicle size at weaning was divided into three classes: small (< 3.5 mm), medium (3.5 - 4.5 mm) or large (≥ 4.5 mm) and tested for relations with reproductive parameters. Nine animals had a follicle size larger than 9 mm at the start of the follicular phase and eight of those did not show estrus. Therefore, 9 mm was considered a threshold for abnormal follicular development. These follicle data were not included in the analyses unless stated otherwise.
Normality of parameters was checked with the UNIVARIATE procedure of SAS. The normally distributed parameters: follicle size at different days and onset and duration of estrus were analysed with the GLM procedure of SAS with treatment as the main effect. For each of the parameters, relevant co-variates: farrowing weight sow, lactational weight loss, weaning weight, backfat and lactational backfat loss were considered as well as the interaction with treatment. If they were found not to be significant, covariates and interactions were omitted from the model. Lactation length was included for all analyses but omitted from the model when not significant. When main effects were significant, means were compared by the least significant difference method adjusted for multiple comparisons according to Bonferroni.

Treatment effects on litter size were analyzed using the GENMOD procedure of SAS with a Poisson distribution. Relations between parameters were tested by calculating the Pearson correlation coefficients. Treatment effect on pregnancy rates and farrowing rates was analyzed using the FREQUENCY procedure in SAS for a χ^2 distribution. A probability value of less than 0.05 was considered significant and $P < 0.1$ was considered a tendency. Data are presented as means ± SD unless stated otherwise.

3.3 Results

3.3.1 Follicular development and estrus

Follicle size at weaning was on average 3.7 ± 1.0 mm and varied from 1.4 to 9.8 mm between animals (Table 1). Average follicle size increased ($P < 0.05$) during altrenogest treatment, resulting in larger follicles at the start of the follicular phase for RU4, RU8 and RU15 compared with controls (5.3 ± 0.9, 5.5 ± 1.3, 5.1 ± 1.2 and 3.4 ± 0.6 mm, respectively; Table 1). Additionally, 4 RU8 sows and 5 RU15 sows had a follicular size of more than 9 mm at the start of the follicular phase (15.7 ± 6.3 mm). Follicle size increased to 19.4 ± 3.1 mm on d 4 of the follicular phase and 8 of the 9 sows subsequently did not show estrus.

At d 4 of the follicular phase, the difference in follicular size between treated and control sows had become smaller, and control sows tended only to differ from RU15 sows (6.8 ± 2.2 *vs.* 7.6 ± 1.0 mm, respectively; $P = 0.1$, Table 1). However, 1 control sow had a follicle size of 19.6 mm on d 4 of the follicular phase, but she did show estrus and was found non-pregnant on d 30 after insemination. Without this sow average follicular size on d 4 of the follicular phase was 6.5 ± 1.1 mm for controls, which differed ($P < 0.05$) from RU8 and RU15, but did not differ ($P > 0.1$) from RU4.

Table 1. Follicle size in mm (mean ± SD and range) for control, RU4, RU8 and RU15 sows

Item	Treatment[1]			
	Control	RU4	RU8	RU15
No. assigned	45	56	53	43
Weaning	3.4 ± 0.6	3.8 ± 1.1	3.8 ± 0.9	3.7 ± 1.1
After 7 days of treatment	-	-	-	5.4 ± 1.5
Start follicular phase	3.4 ± 0.6[a]	5.3 ± 0.9[b]	5.5 ± 1.3[b]	5.1 ± 1.2[b]
Growth during treatment		1.5 ± 1.3	1.6 ± 2.0	1.7 ± 2.0
Day 4 of the follicular phase[3]	6.8 ± 2.2[x]	7.2 ± 0.9	7.4 ± 1.6	7.6 ± 1.0[y]

[1] Treatments involved no altrenogest treatment (control), 20mg of altrenogest Day -1 till Day 2; Day 0 = weaning (RU4), 20mg of altrenogest Day -1 till Day 6 (RU8) and 20mg of altrenogest, Day -1 till Day 13 (RU15).

[ab] means within a row without a common superscript number are different $P < 0.05$

[xy] means within a row without a common superscript number tended to be different for $P = 0.1$

The percentage of sows showing estrus within 10 d after start of the follicular phase was similar (89 to 92%) for the 4 treatments and duration of estrus was also not affected by treatment (Table 2). A total of 25 sows did not show estrus within 10 d after the start of the follicular phase. These consisted of 8 of the 9 animals with follicles larger than 9 mm at the start of the follicular

phase, an additional 2 of 2 animals with follicles larger than 9 mm at d 4 of the follicular phase (1 in control and 1 in RU8), and another 15 of 248 animals (7 in control, 5 in RU4, 2 in RU8 and 1 in RU15) with smaller follicles at both times (5.0 ± 1.4 and 6.1 ± 0.6 mm at the start of the follicular phase and on d 4 of the follicular phase, respectively).

Table 2. Estrus, pregnancy rate, farrowing rate and litter sizes for control, RU4, RU8 and RU15 sows

Item	Treatment[1]			
	Control	RU4	RU8	RU15
No. assigned	71	62	65	61
Estrus				
Estrus within 10 days (%)	89	92	91	90
Onset of estrus (d)2	4.9 ± 1.2^{ax}	3.5 ± 1.1^{b}	4.3 ± 1.7^{y}	3.8 ± 0.9^{b}
Duration of estrus (h)2	59 ± 11	58 ± 11	60 ± 13	62 ± 13
Reproductive performance				
Pregnancy rate on Day 30 (%)3	91	91	80^{x}	95^{y}
Farrowing rate (%)4	89	88	76^{a}	95^{b}
Total number of piglets born	11.9 ± 3.5^{a}	12.1 ± 3.0^{a}	12.0 ± 4.3^{a}	14.4 ± 3.0^{b}
Number of piglets born dead	0.6 ± 1.0	0.5 ± 0.7	0.5 ± 0.8	0.7 ± 1.1
Number of piglets born alive	11.0 ± 3.0^{a}	11.4 ± 2.9^{x}	10.9 ± 3.7^{a}	12.9 ± 2.8^{by}
Average weight of piglets (kg)	1.70 ± 0.25^{x}	1.66 ± 0.17^{x}	1.70 ± 0.30^{x}	1.54 ± 0.18^{y}

[1] Treatments involved no altrenogest treatment (control), 20mg of altrenogest Day -1 till Day 2; Day 0 = weaning (RU4), 20mg of altrenogest Day -1 till Day 6 (RU8) and 20mg of altrenogest, Day -1 till Day 13 (RU15).

[2] Of sows that showed estrus within 10 days

[3] No. of pregnant sows / no. of inseminated sows x 100%

[4] No. of farrowing sows / no. of inseminated sows x 100%

[ab] means within a row without a common superscript number are different $P < 0.05$

[xy] means within a row without a common superscript number tended to be different for $P < 0.1$

3.3.2 Pregnancy rate, farrowing rate and litter size

Pregnancy rate at d 30 was on average 89% overall, but tended ($P < 0.1$) to be greater for RU15 (95%) compared with RU8 (80%; Table 2). Farrowing rate was on average 87% and was greater ($P = 0.04$) in RU15 (95%) than in RU8 (76%). Average litter size was 12.6 ± 3.6 piglets born; RU15 had more piglets (total born and born alive; $P < 0.05$) than the other treatment groups (Table 2).

Table 3. Effects of follicle size at weaning[1] on estrus, pregnancy rate, farrowing rate and litter size for control, RU4, RU8 and RU15 sows[2]

Item						
	Control			RU4		
Follicle size at weaning	Small	Medium	Large	Small	Medium	
All sows						
No. assigned	23	21	0	21	28	
Follicle size at weaning (mm)	2.9 ± 0.4	3.9 ± 0.3	-	3.0 ± 0.4	3.9 ± 0.3	
Showing estrus (%)[4]	91	91	-	95	89	
Farrowing rate (%)[5]	86	84	-	90	88	
Farrowing sows						
No. assigned	18	16	0	18	22	
Litter size (total born)	11.8 ± 3.9	12.7 ± 3.4	-	13.2 ± 3.3	11.8 ± 2.8	

[1] Follicle size at weaning was defined as small; < 3.5 mm, medium; 3.5 - 4.5 mm or large; ≥ 4.5 mm)
[2] Including the 9 sows with a follicle size larger than 9mm at the start of the follicular phase
[3] Treatments involved no altrenogest treatment (control), 20 mg of altrenogest Day -1 till Day 2; Day 0 = weaning (RU4), 20 mg of altrenogest Day -1 till Day 6 (RU8) and 20 mg of altrenogest, Day -1 till Day 13 (RU15).

3.3.3 Follicle size at weaning and its relation to subsequent reproductive performance

Follicular size at weaning was affected by lactational BW loss (loss in kg, r = -0.25; $P = 0.06$; loss in %, r = -0.26; $P = 0.04$). Other lactational parameters (lactation length, weaning weight and backfat at weaning) did not affect follicular size at weaning. Table 3 shows follicular sizes and reproductive performance for control, RU4, RU8 and RU15 sows, with small (< 3.5 mm), medium (3.5 - 4.5 mm) or large (≥ 4.5 mm) follicles at weaning. The percentage of animals showing estrus within 10 d after the start of the follicular phase was not affected by follicular size at weaning (94, 88 and 88%, for sows having small, medium or large follicles, respectively).

Farrowing rates were greater ($P < 0.001$) for sows with small or medium follicles at weaning compared with sows with large follicles at weaning (86 and 78 vs. 48%, respectively). Differences in farrowing rates between follicular size classes were affected by treatment. In controls, farrowing rates were 86 and 84% for sows having small or medium follicles at weaning, respectively. Farrowing rates for treated animals were 90, 88 and 71% in RU4, 94, 83 and 22% ($P < 0.05$) in RU8 and 95, 100 and 83% in RU15 for sows having small, medium or large follicles at weaning, respectively. Litter size was not affected by follicular size at weaning.

For treated animals, a large follicular size at weaning resulted in less follicular growth during treatment (2.6 ± 2.7, 2.3 ± 3.9 and 0.04 ± 2.1 mm for sows having small, medium or large follicles

Treatment[3]						
	RU8			RU15		
Large	Small	Medium	Large	Small	Medium	Large
7	19	27	11	21	18	7
5.8 ± 1.8	2.8 ± 0.6	4.0 ± 0.3	5.1 ± 0.5	2.8 ± 0.6	3.9 ± 0.3	5.4 ± 1.1
100	95	89	82	95	83	86
71	94[a]	83[a]	22[b]	95	100	83
5	17	20	2	19	15	5
11.4 ± 3.3	12.1 ± 5.1	12.2 ± 3.5	12.5 ± 0.7	15.1 ± 3.5	14.3 ± 2.7	14.2 ± 0.8

[4] No. of sows showing estrus within 10 days after start of the follicular phase (10 days after weaning for controls and 11 after last altrenogest for treated animals)/ no. of weaned sows x 100%
[5] No. of farrowing sows /no. of inseminated sows x 100%. Overall significant difference in farrowing rate between small, medium and large follicles at weaning ($P < 0.001$)
[ab] means within a row without a common superscript number are different $P < 0.05$

at weaning, respectively, r = -0.35; $P < 0.01$). Therefore, follicular size at weaning was not related to follicular size at the start of the follicular phase (P > 0.1), but some clear follicle growth patterns were seen. For example, of the RU15 sows that farrowed, sows that had small follicles at weaning (< 3.5mm; mean = 2.8 ± 0.6 mm) showed an increase in follicular size during the first 7 d of treatment to 4.7 ± 1.0 mm and stabilized thereafter to 4.9 ± 1.4 mm (at the start of the follicular phase). The RU15 sows that had medium-size follicles at weaning (≥ 3.5, < 4.5 mm; mean = 4.0 ± 0.3 mm) showed an increase in follicular size during the first 7 d of treatment to 6.2 ± 2 mm and thereafter a decrease to 5.4 ± 1.0 mm (at the start of the follicular phase). The RU15 sows with large follicles at weaning (> 4.5mm; mean = 5.5 ± 1 mm) did not show an increase in follicular size during the first 7 d of treatment to 5.3 ± 3 mm and even had smaller follicles (3.9 ± 1 mm) at the start of the follicular phase.

3.4 Discussion

In this experiment altrenogest was used to extend the interval from weaning to estrus in primiparous sows. In general, positive effects on ovulation rate, pregnancy rate and litter size

are found when the post weaning period is extended by altrenogest treatment after lactation (Morrow et al., 1990; Martinat-Botté et al., 1994; Martinat-Botté 1995; Koutsotheodoros et al., 1998; Patterson et al., 2008). Primiparous sows especially suffer from a negative energy balance at the end of lactation (Zak et al., 1997b; Quesnel et al., 2000), which suppresses follicular development (Quesnel et al., 1998). In these sows, postponement of follicular recruitment by a post weaning altrenogest treatment is believed to restore follicular development, resulting in greater pregnancy rates and larger litter sizes after treatment (Morrow et al., 1990; Martinat-Botté et al., 1994; Martinat-Botté 1995; Koutsotheodoros et al., 1998; Patterson et al., 2008).

In this study, the post weaning period was extended with altrenogest treatments of different durations to evaluate the relation between follicular development during and after altrenogest treatments and to relate this to pregnancy rate and litter size in primiparous sows. It was shown that short altrenogest treatment (4 d) results in similar reproductive performance as in nontreated control sows, an altrenogest treatment for 8 d results in a poorer farrowing rate than control sows and a long altrenogest treatment (15 d) results in superior farrowing rates and litter sizes compared with control sows. Surprisingly, no positive effects on farrowing rate and litter size were found for sows treated for 4 or 8 d. In all altrenogest treatment groups, follicular size increased during treatment, resulting in larger follicles at the start of the follicular phase for treated animals compared with controls. This increase in follicular development is presumably a reflection of greater LH levels during altrenogest treatment than during lactation because decreased LH levels have been associated with reduced follicular size at weaning (Quesnel et al., 1998; Brand et al., 2000). However, the increase may also be related to the positive energy balance during treatment. Quesnel et al., (2007) found a positive relation between follicular development and insulin resistance. Poretsky and Kaling (1987) thought that this was caused by the binding of circulating insulin to ovarian insulin and IGF-1 receptors, thereby interacting with LH and FSH to stimulate follicular development (Poretsky and Kalin, 1987). Van Leeuwen et al., (2010) also found that follicular size increased during altrenogest treatment, which reached a plateau of 5 mm around 6 d of treatment, but no improvement in embryo development on d 5 of gestation was found.

Although the 4 and 8 d altrenogest treatment did result in an improved reproductive performance, this depended on the follicular size at weaning. It appeared that sows with small follicles at weaning (<3.5mm) benefited from a 4- and 8-d altrenogest treatment in terms of farrowing rate. Small follicles at weaning most likely resulted from a heavier lactational burden because sows with small follicular sizes at weaning lost the most BW during lactation and had the least backfat at weaning, which agrees with the findings of other authors (Quesnel et al., 2000; Bracken et al., 2003; Clowes et al., 2003[b]). These results therefore indicate that sows with a more catabolic body condition benefit from a 4- to 8-d post weaning altrenogest treatment, but that less catabolic sows do not.

The finding that sows with large follicles at weaning did not benefit from altrenogest treatment for 4 to 8 d maybe related to LH concentrations during treatment. Redmer and Day (1981) measured relatively low but constant levels of LH during altrenogest treatment, which may result

in the growth of especially the large follicles because large follicles are much more responsive to LH than small follicles (Guthrie 2005). If this is true, then it means that larger follicles at weaning are at risk of being partly recruited for ovulation during treatment and therefore do not become atretic but remain present and grow out to larger sizes after 4 or 8 d altrenogest treatments. These remaining follicles grow to ovulatory size and finally ovulate a relatively "aged" oocyte. In cows, progestagen treatments to synchronize the estrous cycle have been found to prolong the lifespan of the dominant follicle, preventing it from becoming atretic (Sirois and Fortune, 1990). Posttreatment ovulation of this follicle leads to decreased pregnancy rates (Stock and Fortune 1993; Townson et al., 2002). Luteal deficiency cannot be blamed for these decreased pregnancy rates because progesterone concentrations after ovulation of a dominant follicle have been shown to be similar to progesterone concentrations in untreated cows (Stock and Fortune 1993). The negative effects of a prolonged follicular lifespan are more likely related to decreased maturation and fertilization capacities of the oocyte (Revah and Butler, 1996) that compromise fertilization rates, embryo survival or both. Thus, if larger follicles under altrenogest treatment do not turn over this may similarly lead to overmatured oocytes in pigs resulting in decreased farrowing rates and litter sizes. Surprisingly, no effect of follicular size at the start of the follicular phase on subsequent reproduction was found. However, a follicular size that was too large at the start of the follicular phase was not beneficial for subsequent reproductive performance because all 9 sows (4 RU8 and 5 RU15) that had a follicle size of more than 9 mm at the start of the follicular phase had cystic ovaries 4 d later; only 1 of those sows showed estrus and none farrowed. This lack of estrus display in cystic animals maybe explained by an early shift from estrogen to progesterone production, because the development of cysts after weaning has been associated with an early rise in progesterone secretion (Lucy et al., 2001).

There is no good explanation for the fact that there were no control sows with large follicles at weaning because there was no difference in BW loss or backfat depth loss during lactation between treatments. Only 80% of all sows were scanned, but this does not explain the lack of control sows with large follicles at weaning. Therefore, unfortunately, no comparisons between reproductive performance of control sows with large follicles at weaning and treated sows with large follicles at weaning could be made.

In sows treated for 15 d with altrenogest, follicle growth patterns during treatment differed depending on the follicular size at weaning. Small follicles at weaning had increased in size by d 7 and further increased to d 14, whereas large follicles at weaning had decreased by d 7 and further decreased to d 14. In a previous study (Van Leeuwen et al., 2010) daily ultrasound during 15 d of altrenogest treatment showed that the largest 5 follicles increased in size during the first 6 d and, after a small decrease in size at d 7 to 8, remained more or less stable for the remainder of the period. This was thought to be indicative for the occurrence of synchronized waves of follicular growth under altrenogest treatment, similar to the ones observed by Lucy et al. (2001) during lactation. Lucy et al. (2001) described cohorts of 20 to 30 follicles that developed from 2 mm to 4 to 6 mm and regressed after which new follicles started to develop. If indeed follicles turnover during extended altrenogest treatment, this may be an explanation for improved reproductive

performance in the long-treatment group (RU15) for sows with large follicles when treatment was initiated at weaning.

In accordance with the findings of Patterson et al. (2008) 15-d altrenogest treatment (RU15) resulted in the best reproductive results. An explanation for this improved performance may be found in the improved nutritional status of these sows during pre-ovulatory follicular development. The RU15 sows were inseminated on average 19 d post weaning versus 12, 8 and 5 d post weaning for RU8, RU4 and controls, respectively. Such an increase in recovery time improves reproductive performance (Morrow et al., 1990; Clowes et al., 1994; Vesseur et al., 1997) due to improved follicle and oocyte quality and subsequent luteal quality and embryo survival (Zak et al., 1997[ab]; Cosgrove, 1998). Cosgrove (1998) called this improvement in oocyte quality 'nutritional imprinting of oocytes' resulting in better quality oocytes, which are essential for good embryo development and thus embryo survival to maintain litter size (Webb et al., 2007).

In conclusion, this study showed that post weaning altrenogest treatment in primiparous sows increased follicular size, resulting in larger follicles at the start of the follicular phase, and also improved reproductive performance (increased farrowing rate and litter size) in sows treated for 15 d. It also provided evidence that shorter treatment with altrenogest (4 or 8 d) is beneficial for sows with compromised follicular development (small follicles at weaning) but is not recommendable for sows with large follicles at weaning, because treatment of the latter results in a lower farrowing rate.

The changes in follicular size during treatment may indicate the occurrence of follicular turnover during 15 d of altrenogest treatment, resulting in improved reproductive performance for sows with large follicles at the start of treatment. Therefore, positive results from post weaning altrenogest treatment should only be expected in sows with small follicles at weaning unless the duration of treatment is long enough to ensure turnover of the follicle pool present at weaning.

3.5 Acknowledgements

The authors thank Intervet Schering Plough Animal Health and Janssen Animal Health for their financial support, Isowean S.A. and its employees for their participation in this study, Dr. Pablo Villafañe for finding the swine facilities, student Anniek Peek for her help and Intervet SPAH Brasil for supplying the Regumate.

Chapter 4

Effects of altrenogest treatments before and after weaning on follicular development, farrowing rate, and litter size in sows

J.J.J. van Leeuwen*, M.R.T.M. Martens§, J. Jourquin‡,
M.A. Driancourt†, B. Kemp*, N.M. Soede*

*Adaptation Physiology Group,
Department of Animal Sciences,
Wageningen University, the Netherlands
§ Intervet Schering Plough Animal Health, the Netherlands
‡ Janssen Animal Health, Belgium
† Intervet Schering Plough Animal Health, France

Journal of Animal Science (2011) pp 2397-2406

Abstract

In a previous study, we showed that follicle size at weaning affects the response of a sow to a short-term altrenogest treatment after weaning. In this study, an attempt was made to prevent growth of follicles into larger size categories before weaning using different altrenogest treatments before weaning to improve reproductive performance after postweaning altrenogest treatments. Sows (87 primiparous and 130 multiparous) were assigned to the following treatments: control (no altrenogest treatment; n = 59), RU0-20 (20 mg altrenogest, d -1 to d 6; weaning = d 0; n = 53), RU40-20 (40 mg altrenogest, d -3 to d 0 and 20 mg altrenogest d 1 to d 6; n = 53), and RU20-20 (20 mg altrenogest, d -3 to d 6; n = 52). Follicle size was assessed daily with trans-abdominal ultrasound. Follicle size on d -3 (3.6 ± 0.7 mm) and at weaning (4.0 ± 0.7 mm) was similar for all treatments. Altrenogest-treated sows had larger follicles at the start of the follicular phase than control sows (5.4 ± 0.1 and 3.8 ± 0.2 mm, LS Means, respectively; $P < 0.0001$) and on d 4 of the follicular phase (8.0 ± 0.1 and 6.7 ± 0.2 mm, LS Means, respectively; $P < 0.0001$). Multiparous sows had larger follicles than primiparous sows at the start of the follicular phase (5.3 ± 0.1 and 4.7 ± 0.1 mm, LS Means, respectively; $P < 0.01$) and on d 4 of the follicular phase (8.0 ± 0.1 and 7.0 ± 0.1 mm, LS Means, respectively; $P < 0.0001$). Farrowing rate and litter size (born alive + dead) were not affected by treatment or parity. However, in primiparous sows, when mummies were included in litter size, altrenogest sows had larger litters than control sows (13.4 ± 0.5 and 11.9 ± 0.7 piglets, respectively; $P = 0.02$). In primiparous control sows, backfat depth at weaning and litter size were positively related (slope of the regression line = 0.82; $P < 0.05$), which was not the case in primiparous altrenogest sows. In conclusion, the different altrenogest treatments before weaning did not prevent growth of follicles before weaning and similarly affected subsequent follicle development and fertility. In primiparous sows, altrenogest treatment after weaning increased the number of fetuses during pregnancy, but positive effects seemed limited by uterine capacity. Altrenogest treatment after weaning improved litter size in primiparous sows with reduced backfat depth at weaning, which suggests a specific positive effect of a recovery period after weaning in sows with reduced BCS at weaning.

Key words: altrenogest, farrowing rate, follicle development, litter size, sow

4.1 Introduction

Reduced reproductive performance in the second litter as a result of a negative energy balance during first lactation can be prevented by altrenogest treatments after weaning. Several studies, differing in timing, duration, and dose of altrenogest treatments have found greater ovulation rates (Koutsotheodoros et al., 1998; Patterson et al., 2008), better synchronization of estrus (Martinat-Botté et al., 1994; Martinat-Botté, 1995), improved embryo survival (Patterson et al., 2008), and greater litter sizes (Martinat-Botté et al., 1990; van Leeuwen et al., 2010) for primiparous sows treated with altrenogest after weaning.

The positive effect of altrenogest treatment after weaning on subsequent reproductive performance seems dependent on the duration of treatment in relation to follicular status at weaning. Van Leeuwen et al. (2011) found that a postweaning altrenogest treatment for 15 consecutive days resulted in improved reproductive performance (increased farrowing rate and litter size) regardless of follicular status at weaning, but shorter postweaning altrenogest treatments (4 or 8 d) were only beneficial for sows with small follicles at weaning, and adversely affected fertility in sows with large follicles at weaning. It was hypothesized that these adverse effects were caused by "aging" of the larger follicles that failed to go into atresia during short altrenogest treatments (van Leeuwen et al., 2011[a]).

Therefore, this study used altrenogest treatments before weaning of different doses in an attempt to prevent growth of follicles before weaning, and it investigated the consequences on reproductive performance after subsequent postweaning altrenogest treatments.

4.2 Materials and Methods

The study was conducted during spring, from September 2009 to December 2009 in a commercial swine herd in Monte Buey, Argentina (32°25'00"S, 62°27'00"W) following the Argentine animal welfare law (Ley penal 14346).

4.2.1 Animals, housing, and diets

Pregnant sows (n = 217; line PIC23) entered the maternity facilities at 2 to 6 d before farrowing, at approximately 319 to 323 d of age, weighing an average 246 ± 29 kg. On d 114 of gestation, all sows received 75µg intramusculary of a $PGF2_{\alpha}$ analog (D cloprostenol, Bioprost, Biotay, Buenos Aires, Argentina). At farrowing, the numbers of piglets born alive or dead were recorded, and the total weight of piglets born alive and dead was assessed. Litter size (total born) averaged 12.6 ± 2.7 piglets. Postpartum cross fostering was performed within 3 d to adjust litter size to 11 to 13 piglets. During lactation, sows were fed a corn- and soybean-based diet, formulated to contain 14.48 MJ DE/kg with 18% CP and 1% lysine, to appetite to a maximum of 8 kg/d. On d 21 (d 19 to 23) of lactation, piglets were weaned (10.8 ± 1.1 piglets/sow) and sows were moved to individual crates in another barn and fed once each day (0700 h) to a maximum of 4 kg of a corn- and

soybean-based diet formulated to contain 13.65 MJ/kg with 14% CP and 1% lysine. All crates were equipped with individual drinking nipples providing water ad libitum.

Daily minimum and maximum environmental temperatures were registered throughout the experiment. Average temperature varied between maximum of 20.0 ± 5°C and a minimum of 6.6 ± 4°C in September and a maximum of 27.7 ± 3°C and a minimum of 17 ± 3°C in December. When the environmental temperature (outside the barn) exceeded 28°C, spray coolers were activated in the gestation barn.

Sows were weighed and P2 backfat depth was measured using A-mode ultrasound (Lean-Meater, Renco Corporation, Minneapolis, MN) when entering the farrowing facilities, at weaning and for altrenogest-treated sows on the day after last altrenogest treatment. Farrowing weight was corrected for weight of the litter at birth + estimated placental weight, amnion weight (216 g/fetus), and for estimated fetal growth during the time between weighing and farrowing (60 g/fetus/d) based on Leenhouwers et al. (2002) and van Rens et al. (2002, 2005). Corrected farrowing weight averaged 227 ± 28 kg and backfat depth at farrowing was 18.0 ± 7 mm.

4.2.2 Treatments

Sows [primiparous: n = 87; multiparous (parity 2 to 3): n = 130] were randomly assigned to 1 of 4 treatments (weaning = d 0): control (no altrenogest treatment; 30 primiparous and 29 multiparous sows), RU0-20 (20 mg altrenogest, d-1 to d 6; 16 primiparous and 37 multiparous sows), RU40-20 (40 mg altrenogest, d -3 to d 0 and 20 mg altrenogest d 1 to d6; 23 primiparous and 30 multiparous sows) and RU20-20 (20 mg altrenogest, d -3 to d 6; 18 primiparous and 34 multiparous sows). Altrenogest (20 mg = 5 mL Regumate, Intervet SPAH, Huixquilucan, Estado de Mexico, Mexico), a progesterone analog, was administered daily as a top dressing over a small portion of the feed to ensure ingestion of the entire dose.

4.2.3 Estrus detection and insemination

From weaning onwards, estrus detection was performed daily at 1030 h by a trained farm technician using fence line boar contact. Estrus was considered to start when a sow first exhibited a standing reflex during a back pressure test in the presence of a boar and to end when a sow first failed to exhibit a standing reflex. Sows were artificially inseminated in the afternoon after the first detected estrus (1400 h), and inseminations were repeated every next morning at 0830 h, if the sow still exhibited a good standing reflex. Inseminations consisted of a 100 mL dose of semen that was obtained from the farm's boars (PIC 415), with a dosage of 4 x 10^9 sperm cells. Merck III (Mini Tüb, Tiefenbach, Germany) was used as extender. Semen was stored at 17°C for a maximum of 3 d.

4.2.4 Follicular development and reproductive performance

Transabdominal ultrasound with a 5-MHz multiconvex transducer (Aquila, Piemedical, Maastricht, The Netherlands) was performed to assess follicular development. One ovary was scanned each time because it was considered to be representative of the contra lateral ovary (N.M.

Soede; unpublished results). Recorded images were subsequently reviewed using the cineloop application of the scanner. The diameters of the 5 largest follicles were measured and averaged.

Follicular development was assessed on d -3 for controls, RU40-20 and RU20-20, at d 0 (weaning) for all sows, 1 d after last altrenogest treatment for altrenogest-treated sows, and on d 4 of the follicular phase for all sows. The onset of the follicular phase was assumed to start at weaning for control animals and 1 d after last altrenogest administration for altrenogest-treated animals (van Leeuwen et al., 2010; 2011).

On d 114 of gestation, pregnant sows received 75 µg intramuscularly of a $PGF2_{\alpha}$ analog (D cloprostenol, Bioprost). At farrowing, piglets were weighed and the numbers of piglets born alive or dead were recorded and placentas were thoroughly checked for (hidden) mummies, after which total number of mummies was recorded.

4.2.5 Statistical analyses

Data were analyzed using SAS (SAS Inst. Inc., Cary, NC). Sows that failed to show estrus within 10 d after weaning (4 out of 59 control sows) or 11 d after last altrenogest treatment (4 out of 158 altrenogest-treated sows) were marked as not showing estrus and their follicular development and subsequent reproductive performance were not included in the analyses. Normality of the model residuals was checked with the UNIVARIATE procedure of SAS. The normally distributed parameters were analyzed with the GLM procedure of SAS with treatment + parity class as main effects plus their interaction (model 1). When the interaction was not significant, it was omitted from the model. Whenever main effects where significant, means were compared by the LSM method adjusted for multiple comparisons according to Bonferroni.

Because none of the reproductive performance parameters differed among the 3 altrenogest treatments, analyses for treatment effects on subsequent fertility were also performed on pooled data from all altrenogest-treated sows vs. control sows.

To study the effects of lactational burden with follicular development and litter size, model 1 was extended with backfat depth at weaning, backfat depth loss, BW at weaning or BW loss during lactation, and their interactions with treatment or parity (model 2).

Follicular development was further analyzed with the GLM procedure of SAS using repeated measures. The GLM model included treatment, parity (primiparous or multiparous), sow within treatment x parity, and day of the follicular phase (start of follicular phase = d 0 for controls and d 7 for altrenogest-treated animals, or fourth day of the follicular phase). Differences in follicle size between treatments and between parities were tested against the error term of sow within treatment x parity, and differences in follicle size between days of the follicular phase were tested against the overall error term. The interaction between treatment and day of the follicular phase and between treatment and parity were not significant and therefore were omitted from the model.

Treatment effects on estrous rate (percentage of sows showing estrus within 10 d from weaning or 11 d from last altrenogest treatment) and farrowing rate were analyzed using the FREQUENCY procedure in SAS with a Fisher's exact test. Synchrony of estrus was expressed as % of sows in estrus within a 48-h period. For primiparous sows (controls and altrenogest-

treated), the 48-h time window covered d 4 to 5 after start of the follicular phase. For multiparous altrenogest-treated sows and control sows respectively, the tightness of synchronization was measured on d 3 to 4 or d 4 to 5 after start of the follicular phase.

A probability level of < 0.05 was considered significant and $P < 0.1$ was considered a tendency. Data presented in the text are means ± SD unless stated otherwise, and data presented in the tables are least square means ± SE.

4.3 Results

4.3.1 Lactation, BW loss, and backfat depth loss

From 20.6 ± 1.6 d of lactation, sows lost on average 19.3 ± 17.6 kg of BW. This was 8.3 ± 7.6% of their BW and 3.7 ± 2.1 mm of backfat depth, which was similar for all treatments. Primiparous sows differed from multiparous sows in farrowing weight [203 ± 2 and 243 ± 2 kg (least square means), respectively; $P < 0.0001$) and number of piglets weaned [10.6 ± 0.1 and 11.0 ± 0.1 piglets (least square means), respectively; $P < 0.008$], and tended to have lost less backfat depth [3.4 ± 0.2 and 3.9 ± 0.2 mm (least square means), respectively; $P < 0.06$].

4.3.2 Follicular development

Two sows showed abnormal follicular development and their data were excluded from the analyses on follicular development: 1 primiparous sow in the RU20-20 had a follicle size of 16 mm at weaning and of 9 mm at the start of the follicular phase. She showed estrus and subsequently

Table 1. Follicle size in mm (LS Means ± SE) for control, RU0-20, RU40-20, and RU20-20 and primiparous or multiparous sows

Item	Treatment[1]			
	control	RU0-20	RU40-20	RU20-20
No. assigned	55	49	53	50
d -3	3.5± 0.1	-	3.6 ± 0.1	3.6 ± 0.1
d 0 (weaning)	3.9 ± 0.1	4.1 ± 0.1	3.9 ± 0.1	4.1 ± 0.1
Start follicular phase[2]	3.9 ± 0.2[a]	5.4 ± 0.2[b]	5.3 ± 0.2[b]	5.3 ± 0.2[b]
d 4 follicular phase	6.7 ± 0.2[a]	8.0 ± 0.2[b]	7.7 ± 0.2[b]	7.7 ± 0.2[b]
Growth d -3 to d 0	0.5 ± 0.2	-	0.2 ± 0.2	0.7 ± 0.2
Growth rate d -3 to d 0[3]	0.11 ± 0.06	-	0.07 ± 0.06	0.21 ± 0.06
Growth during treatment[4]	-	1.4 ± 0.3	1.7 ± 0.3	1.2 ± 0.3
Growth rate after weaning[5]	0.72 ± 0.03[a]	0.16 ± 0.03[b]	0.20 ± 0.03[b]	0.15 ± 0.03[b]

delivered 15 piglets. One multiparous sow in the RU0-20 treatment had a follicle size of 19 mm at d 4 of the follicular phase, showed estrus, but was found non-pregnant on d 30.

Follicle size on d -3 was similar for control, RU40-20, and RU20-20 (on average 3.6 ± 0.7 mm) and was not affected by parity (Table 1). From d -3 until weaning, follicles grew on average 0.5 ± 0.9 mm (0.15 ± 0.3 mm/d), which resulted in an average follicle size at weaning of 4.0 ± 0.7 mm. Follicular growth rate between d -3 and d 0 was less in primiparous than in multiparous sows (0.05 ± 0.06 and 0.21 ± 0.04 mm/d, respectively; $P = 0.03$). Follicle size at weaning was similar among treatments, but primiparous sows had smaller follicles at weaning than multiparous sows (3.8 ± 0.1 and 4.1 ± 0.1 mm, respectively; $P = 0.004$, Figure 1). During altrenogest treatment, follicles grew 1.6 ± 2.1 mm, resulting in larger follicles at the start of the follicular phase for altrenogest-treated animals compared to control (5.4 ± 0.2, 5.3 ± 0.2, 5.3 ± 0.2, and 3.9 ± 0.2 mm, for the RU0-20, RU40-20, RU20-20 and control treatments, respectively; $P < 0.0001$, Table 1 and Figure 1). Overall, follicle size was smaller at the start of the follicular phase than on d 4 of the follicular phase (4.9 ± 0.1 and 7.7 ± 0.1 mm, LS means, respectively; $P < 0.0001$), was smaller in control sows than in altrenogest-treated sows (5.3 ± 0.1, 6.7 ± 0.2, 6.5 ± 0.2 and 6.6 ± 0.2 mm, LS Means for control, RU0-20, RU40-20, and RU20-20, respectively; $P < 0.0001$), smaller in primiparous sows than in multiparous sows (5.9 ± 0.1 and 6.7 ± 0.1 mm, LS Means, respectively; $P < 0.0001$), and there were no significant interactions among day, treatment, and parity. When comparing the pooled data of altrenogest-treated sows with control sows, follicle size at the start of the follicular phase was larger for altrenogest-treated sows than for control sows (5.4 ± 0.1 and 3.8 ± 0.2 mm, LS Means, respectively; $P < 0.0001$).

	Parity	
	1	2 to 3
	82	125
	3.6 ± 0.1	3.6 ± 0.1
	3.8 ± 0.1[a]	4.1 ± 0.1[b]
	4.7 ± 0.1[a]	5.3 ± 0.1[b]
	7.0 ± 0.1[a]	8.0 ± 0.1[b]
	0.3± 0.2[x]	0.6 ± 0.1[y]
	0.05 ± 0.06[a]	0.21 ± 0.04[b]
	1.0 ± 0.3	1.9 ± 0.2
	0.27 ± 0.03[x]	0.34 ± 0.02[y]

[ab] means within a row without a common superscript number are differ ($P < 0.05$).

[xy] means within a row without a common superscript number tended to differ ($P = 0.1$).

[1] Treatments involved no altrenogest treatment (control), 20mg of altrenogest d -1 to d 6; d 0 = weaning (RU0-20), 40 mg of altrenogest d -3 to d 0 and 20 mg of altrenogest d 1 to d 6 (RU40-20) and 20 mg of altrenogest, d -3 to d 6 (RU20-20). Sows that did not show estrus within 10 d after start of the follicular phase were excluded.

[2] start follicular phase = weaning for control sows and 1 d after last altrenogest administration for altrenogest treated sows.

[3] Average growth rate (mm/d) from d -3 to d 0.

[4] Treatment*Parity ($P = 0.04$): 0.7 ± 0.5 (RU0-20/Primiparous), 2.2 ± 0.3 (RU0-20/Multiparous), 2.0 ± 0.4 (RU40-20/Primiparous), 1.5 ± 0.4 (RU40/Multiparous), 0.5 ± 0.5 (RU20-20/Primiparous) and 1.9 ± 0.3 (RU20-20/Multiparous) mm, LS Means ± SE.

[5] Average growth rate (mm/d) from weaning until d4 of the follicular phase for controls and from weaning until start of the follicular phase for altrenogest treated.

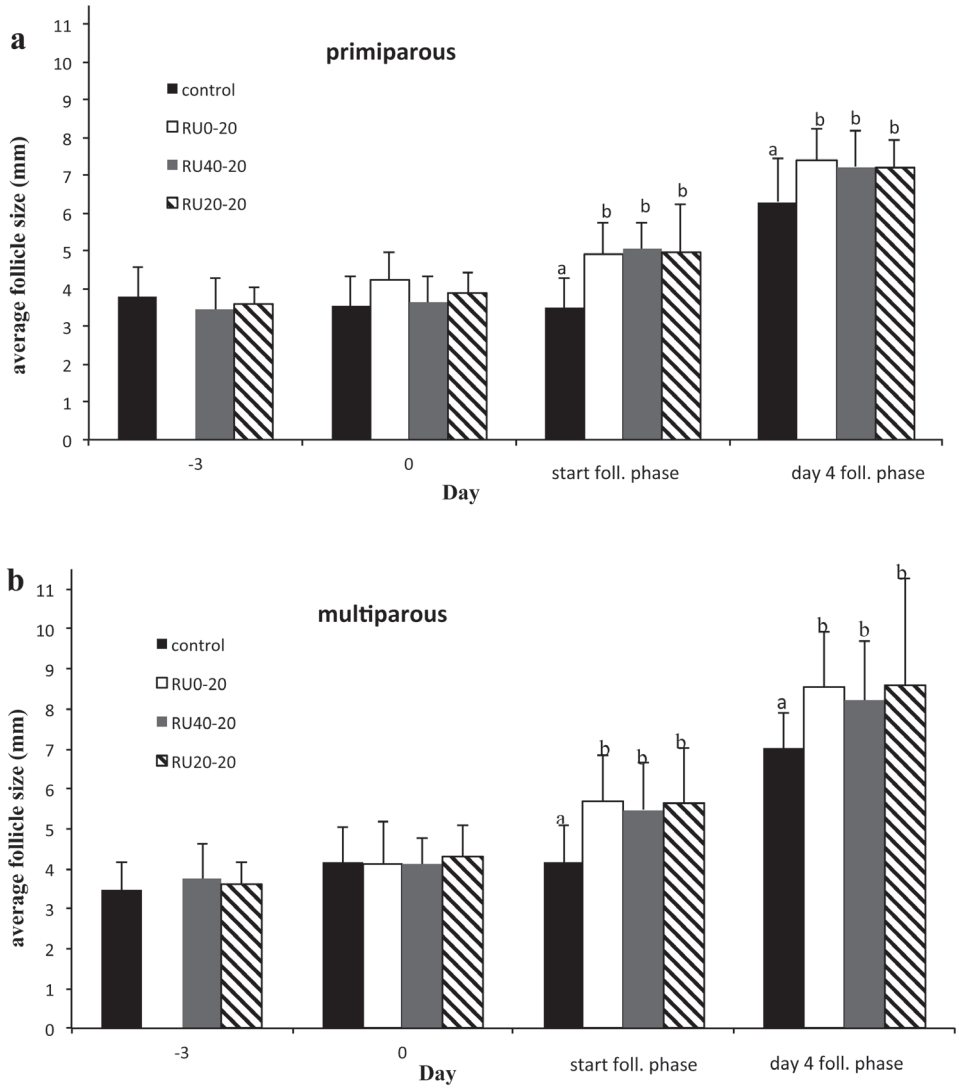

Figure 1. Follicular development (means ± SD) in primiparous (a) and multiparous (b) sows for control (no altrenogest, RU0-20 (20 mg of altrenogest d-1 to d6), RU40-20 (40 mg of altrenogest d-3 to d0 and 20 mg of altrenogest d1 to d6), and RU20-20 (20 mg of altrenogest d-3 to d6). Sows that did not show estrus within 10 d after start of the follicular phase were excluded. The follicular phase started at weaning for controls and 1 d after last altrenogest administration for treated animals.
[ab] Means within a day without a common superscript number differ ($P < 0.05$).

On d 4 of the follicular phase, average follicle size was still larger for altrenogest-treated sows as compared with control sows (8.0 ± 0.1 and 6.7 ± 0.2 mm, respectively; $P < 0.0001$), and was similar for all altrenogest treatments (8.0 ± 0.2, 7.7 ± 0.2, and 7.7 ± 0.2 mm, for RU0-20, RU40-20, and RU20-20, respectively; $P = 0.4$). The follicular growth pattern was similar among parities but primiparous sows had smaller follicles than multiparous sows at the start of the follicular phase (4.7 ± 0.1 and 5.3 ± 0.1 mm, respectively; $P = 0.009$) and on d 4 of the follicular phase (7.0 ± 0.1 and 8.0 ± 0.1 mm, respectively; $P < 0.0001$, Figure 1; d 4 of the follicular phase = d 11 in Figure 1). Follicles of altrenogest-treated sows grew on average 0.18 ± 0.18 mm/d during altrenogest treatment versus 0.72 ± 0.27 mm/d from weaning until d 4 of the follicular phase for control sows ($P < 0.0001$).

4.3.3 Estrus, farrowing rate, and litter size

On average, 96% of the sows showed estrus within 10 d after the start of the follicular phase. In primiparous sows this percentage was lower in control sows than in altrenogest-treated sows (87% and 100%, respectively; $P = 0.01$). Estrus started on average 4.4 ± 1.1 d after start of the follicular phase. In primiparous sows the interval to estrus was not affected by treatment, but multiparous, altrenogest-treated sows had shorter intervals to estrus than control sows (3.6 ± 0.1 and 4.3 ± 0.2 d, respectively; $P = 0.001$, Table 2). In primiparous sows, estrus tended to be more synchronized (% of sows in estrus within a 48-h period) in RU0-20 (88%) and RU20-20 (89%) compared to RU40-20 (78%) and control (63%; $P = 0.1$; Figure 2a). In multiparous sows, synchrony of estrus was not affected by treatment (Figure 2b). Duration of estrus was similar among treatments and lasted on average 63 ± 13 h, but was shorter in primiparous sows than in multiparous sows (59 ± 2 and 65 ± 1 h, respectively; $P = 0.007$, Table 2).

Farrowing rate was on average 87% and was not affected by treatment or parity (Table 2). In primiparous sows, litter size averaged 12.3 ± 2.5 piglets (born alive + dead), and altrenogest-treated sows had on average 0.9 more piglets per litter than control sows, although this increase failed to reach significance ($P = 0.16$; Table 2). However, when mummies were included in litter size, primiparous altrenogest-treated sows had larger litters than primiparous control sows (13.4 ± 0.5 and 11.9 ± 0.7 piglets, respectively; $P = 0.02$). In contrast, in multiparous sows, treatment did not affect litter size (13.2 ± 0.4 and 13.7 ± 0.6 piglets, for altrenogest-treated sows and control sows, respectively). Total litter weight was not affected by treatment and primiparous sows had a lower litter weight than multiparous sows ($P < 0.0001$; Table 2).

Table 2. Follicle size in mm, estrus, farrowing rate, and litter size (LS Means ± SE) for control and altrenogest-treated, primiparous and multiparous sows

Parity	Primiparous		Multiparous	
Treatment	control	altrenogest	control	altrenogest
Item	n = 26	n = 56	n = 29	n = 96
Follicle development				
Follicle size at weaning	3.6 ± 0.2	3.9 ± 0.1	4.2 ± 0.1	4.1 ± 0.1
Follicle size start follicular phase2	3.6 ± 0.2^{a}	5.0 ± 0.2^{b}	4.2 ± 0.1^{a}	5.6 ± 0.1^{b}
Follicle size d 4 follicular phase	6.5 ± 0.2^{a}	7.3 ± 0.2^{b}	7.0 ± 0.2^{ab}	8.3 ± 0.1^{c}
Estrus	n = 30	n = 57	n = 29	n = 101
Estrus within 10 d (%)	87^{a}	100^{b}	100^{b}	96^{b}
Onset estrus (d)3	4.8 ± 0.2^{a}	4.8 ± 0.1^{a}	4.3 ± 0.2^{a}	3.6 ± 0.1^{b}
Duration estrus (h)4	59.1 ± 3^{a}	59.4 ± 2^{a}	63.7 ± 2^{ab}	65.8 ± 1^{b}
Synchrony of estrus5	63^{a}	84^{b}	90	77
Reproductive performance	n = 26	n = 57	n = 29	n = 97
Farrowing rate6	88	86	93	86
Total born (alive +dead)	11.7 ± 0.6	12.6 ± 0.4	13.3 ± 0.6	12.8 ± 0.3
Total born + mummies	11.9 ± 0.7^{a}	13.4 ± 0.5^{b}	13.7 ± 0.6^{b}	13.2 ± 0.4^{b}
Born alive	11.0 ± 0.6	12.2 ± 0.4	12.4 ± 0.6	12.1 ± 0.3
Total litter weight (kg)	17.26 ± 0.8^{a}	17.59 ± 0.6^{a}	21.04 ± 0.8^{b}	20.07 ± 0.4^{b}
Average weight / piglet (kg)	1.6 ± 0.5	1.5 ± 0.3	1.6 ± 0.4	1.5 ± 0.02

^{abc} means within a row without a common superscript number differ ($P < 0.05$).

[1] Treatments involved no altrenogest treatment (control) or altrenogest treatment until d6 after weaning (weaning = d0).

[2] start follicular phase = weaning for control sows and 1 d after last altrenogest administration for altrenogest treated sows.

[3] Of sows that showed estrus within 10 d.

[4] Of sows that showed estrus within 10 d. Effect of Parity 59.2 ± 2 and 64.8 ± 1, LS Means ± SE, for primiparous and multiparous, respectively; $P = 0.007$.

[5] % of sows in estrus within 48h period.

[6] No. of farrowing sows / no. of inseminated sows x 100%.

4.3.4 Backfat depth at weaning and litter size

In primiparous sows, an interaction was found between treatment and backfat depth at weaning ($P = 0.02$; Figure 3a) for litter size. Control sows with a greater backfat depth at weaning had a larger litter size (regression line, $y = 0.82x + 0.13$; $P = 0.05$), which was not the case in altrenogest-treated sows (regression line, $y = 0.22x + 9.6$; $P = 0.6$). In multiparous sows, backfat at weaning was not related with subsequent litter size (Figure 3b).

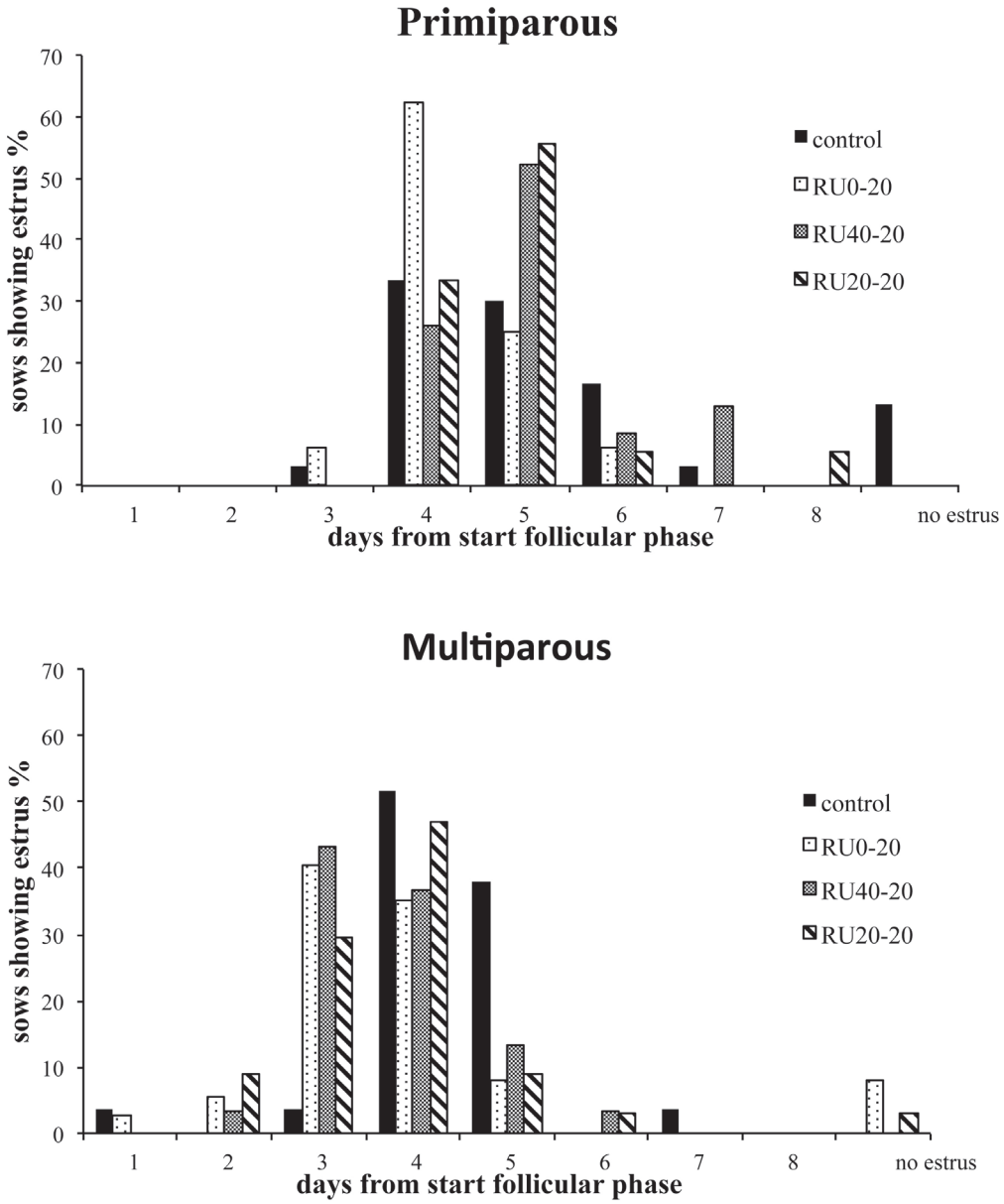

Figure 2. Onset of estrus (days from start of the follicular phase) in primiparous (a) or multiparous (b) sows for control (no altrenogest), Ru0-20 (20 mg of altrenogest d-1 to d6), RU40-20 (40 mg of altrenogest d-3 to d0 and 20 mg of altrenogest d1 to d6), and RU20-20 (20 mg of altrenogest d-3 to d6). The follicular phase started at weaning for controls and 1 d after last altrenogest administration for altrenogest-treated animals.

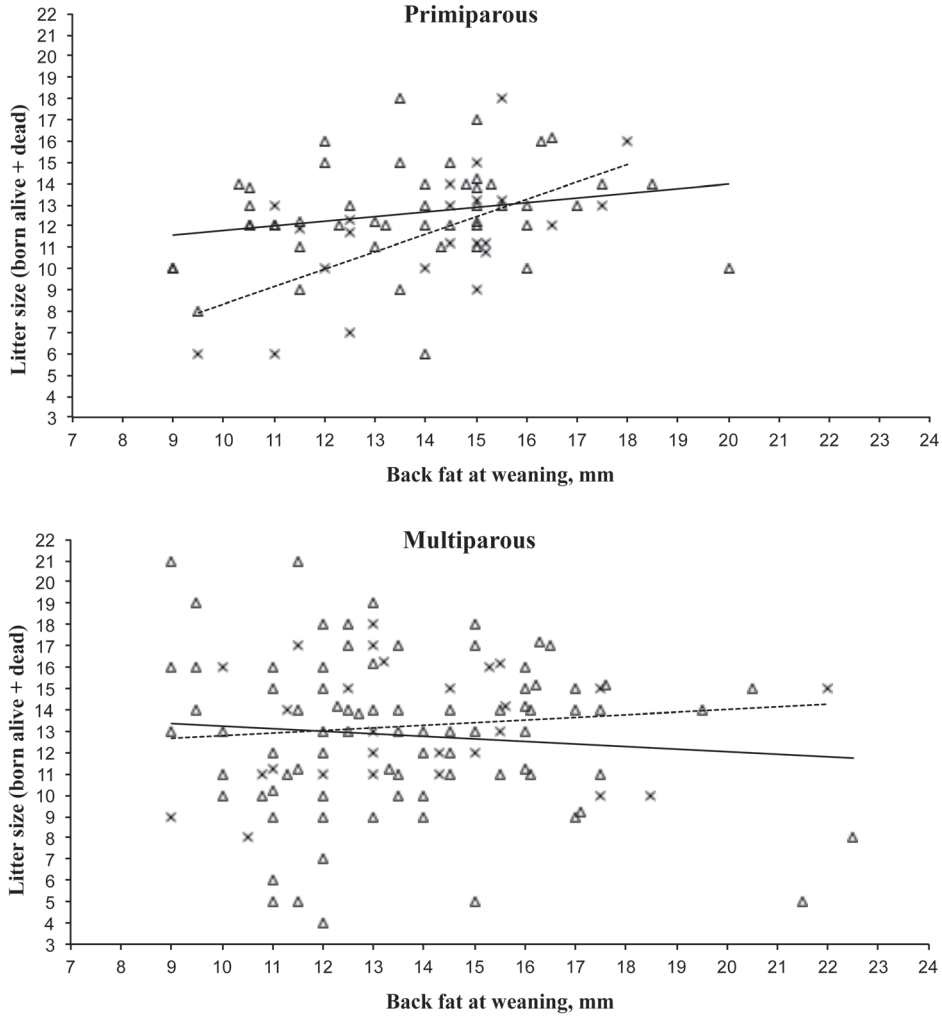

Figure 3. Back fat depth at weaning (mm) and subsequent litter size (born alive + dead) in (a) primiparous sows ----x control (litter size = 0.82 ($P < 0.05$) * back fat + 0.13), and --- Δ altrenogest (litter size = 0.22 ($P > 0.1$) * back fat + 9.6) sows (P (interaction) = 0.02) and (b) multiparous sows ----x control and ---Δ altrenogest (not significant).

4.4 Discussion

In an earlier study (van Leeuwen et al., 2011[a]), we demonstrated that short-term altrenogest treatments after weaning have beneficial effects on reproductive performance only when small follicles are present on the ovaries at weaning. Therefore, in this study an attempt was made to prevent growth of follicles into larger size categories during the last days of lactation using altrenogest treatments before weaning, and to study subsequent fertility after a postweaning

altrenogest treatment. In this study, follicle size increased similarly before weaning in control sows and altrenogest-treated sows, resulting in a similar average follicle size at weaning of 4.0 mm. Follicles become fully LH dependent between 2 to 4 mm (Liu et al., 2000) and it was expected that the synergistic suppressive effects of lactation and altrenogest administration on LH release would stop the growth of the LH-dependent follicles, and possibly induce atresia (as happens during inadequate LH pulsatility in lactation; Lucy et al., 2001). It was surprising, however, altrenogest administration before weaning did not prevent follicular growth before weaning.

Regardless of dose, follicle size increased also during altrenogest treatment after weaning to approximately 5.3 mm at the start of the follicular phase, which is similar to the results of previous studies where follicle size after altrenogest treatment averaged 4.8 mm (van Leeuwen et al., 2010) and 5.3 mm (van Leeuwen et al., 2011[a]). Effects of progestagen treatment on follicular development have been extensively studied in cattle. During progestagen treatments, the frequency of LH pulses reduces, which allows for some follicular development to occur, but prevents further development and ovulation of the dominant follicle in dairy (Burke et al., 1994) and beef (Bo et al., 1995) breeds. Low LH pulsatility, as found during progestagen treatment, is not sufficient to sustain continued growth of the dominant follicle, which as a result, becomes atretic. Therefore, rates of atresia are greater during progestagen treatment (Savio et al., 1993). A similar situation may be happening during altrenogest treatment in sows, where LH pulsatility is reduced. The suppressed LH level may be great enough to sustain follicular development of larger follicles up to a size of about 5 mm, but too small to support final development to pre-ovulatory sizes. Outgrowth of new cohorts of follicles is prevented by inhibin production of the larger follicles. Results from a recent study in our laboratory indicate that LH pulsatility is fully suppressed for the first 12 h after a 20-mg altrenogest administration, after which LH pulsatility progressively resumes (van Leeuwen et al., 2011[b]). Thus, once daily altrenogest administration results in variable intensity of inhibition of LH secretion throughout the day. This may explain why LH-dependent follicles may grow and survive until they reach 5 mm in diameter, but fail to increase further to reach pre-ovulatory sizes.

However, follicle size at weaning did not affect fertility in the present study, which seems to contradict earlier results (van Leeuwen et al., 2011[a]) where farrowing rates were reduced in sows with large follicles at initiation of an 8-d post weaning altrenogest treatment. The current study was conducted on the same farm, under similar conditions, with animals of the same genetics, and after a similar lactational burden. Sows also had similar follicle sizes at weaning and at the start of the follicular phase. It is, therefore, not clear why effects of follicle size at weaning on fertility after altrenogest treatment were not replicated in the present study.

In primiparous sows, altrenogest-treated sows had larger litters (piglets born alive + piglets born dead + mummified fetuses) than did control sows (13.4 vs. 11.9). This is in agreement with studies that found improved ovulation rates after post weaning altrenogest treatment (Koutsotheodoros et al., 1998; Patterson et al., 2008). However, Foxcroft et al. (2006) already demonstrated that when ovulation rates exceed uterine capacity, uterine crowding during the fetal stage of pregnancy results in fetal mortality. Indeed, the fact that litter sizes of primiparous,

altrenogest-treated sows were not significantly larger after exclusion of mummies indicates that fetal mortality rate was indeed increased in altrenogest-treated sows. This agrees with the findings of Patterson et al. (2008) who found that viable embryo numbers decreased between d 30 and d 50 of gestation after post weaning altrenogest treatment, specifically in those sows that had a high ovulation rate. Thus, altrenogest treatments may generate larger litter sizes when uterine capacity is not limiting during the last part of pregnancy.

Primiparous sows are often weaned with a low BCS score associated with suboptimal reproductive performance in the subsequent breeding cycle (Morrow et al., 1989; Zak et al., 1997b). Primiparous sows in this study lost on average 9% of their BW compared to 7% in multiparous sows, also showing that in this study, the lactational burden was greater for primiparous sows. Because BCS at weaning is often related to lactational BW loss (in our study backfat at weaning was related to lactational BW loss in primiparous sows; results not shown), it is not clear which of the two is more limiting for reproductive performance. In this experiment, primiparous control sows with low backfat depth at weaning had a smaller subsequent litter size than primiparous control sows with high backfat depth at weaning; but this effect was absent in sows that received altrenogest treatment. Altrenogest-treated sows were inseminated on average 12 d after weaning, while control sows were inseminated on average 5 d after weaning. So the positive effect of altrenogest administration is most likely to be an effect of the extended recovery period from weaning as provided by altrenogest treatment. This longer post weaning recovery period allows for an improved nutritional status during pre-follicular and pre-ovulatory follicular development, which may improve follicle and oocyte quality and thereby, increase embryo survival (Zak et al., 1997ab; Cosgrove, 1998). Multiparous sows that were treated with altrenogest did not show an increased litter size. This seems to indicate that a recovery period after weaning (during altrenogest treatment) only improves reproductive performance in sows with a compromised body condition at weaning.

In conclusion, the altrenogest treatments before weaning that were used in this study did not prevent an increase in follicle size in the last days before weaning. Altrenogest treatment after weaning resulted in an increased follicle size at the onset of the follicular phase compared to the control animals. Primiparous altrenogest-treated sows overall had larger litters when mummies were included in litter size, but did not have significantly more piglets (born alive + born dead), probably due to a limited uterine capacity. An increase in litter size was seen for altrenogest-treated primiparous sows (but not multiparous sows) with a reduced backfat depth at weaning. This indicates that the longer recovery period after weaning improved litter size in sows with a compromised body condition after lactation, a more common finding in primiparous sows than in multiparous sows.

Acknowledgements

The authors thank Intervet Schering Plough Animal Health and Janssen Animal Health for their financial support, Isowean S.A, Monte Buey, Argentina and its employees for their participation in this study, especially Lujan Mattio for her contribution to the field work, and Intervet SPAH (Huixquilucan, Estado de Mexico, Mexico) for supplying the Regumate.

Chapter 5

Split weaning before altrenogest synchronization of multiparous sows alters follicular development and reduces embryo survival

J.J.J. van Leeuwen[a], M. Verhoeven[a], I. van der Heden-van Noort[a], S. Kranenbarg[b], B. Kemp[a] and N.M. Soede[a],

[a]Adaptation Physiology Group, Department of Animal Sciences, Wageningen University, the Netherlands;
[b]Experimental Zoology Group, Department of Animal Sciences, Wageningen University, the Netherlands;

Accepted by Reproduction of Domestic Animals

Abstract

This study used split-weaning to induce differences in follicle size at weaning and study its consequences for follicle development during and after post weaning altrenogest feeding and for reproductive performance. Multiparous sows (n = 47) were assigned to split-weaning (SW; n = 23; litter size reduced to the six smallest piglets 3 days before weaning) or Control (C; n = 24; normal weaning). Altrenogest (20 mg/day) was fed to all 47 sows from Day -1 till Day 5 (complete weaning = Day 0). Follicle size on Day 1, 2 and 8, was smaller in C than in SW ($P \leq 0.05$). Ovulation rate was similar, but C sows had higher embryo survival rate (ESR) than SW sows (83 ± 19 and 58 ± 31 %, respectively; $P = 0.001$). SW sows with low ESR (<63%; n=10) had a greater follicle size on days 3-6 than SW sows with high ESR (>63%; n=10; $P \leq 0.04$). A decrease in follicle size between Day 5 and 6 of altrenogest feeding was associated with increased ESR in both treatments ($P = 0.002$). Follicle pool analyses (assessment of all follicles >2 mm) revealed that, on Day 3, sows with low ESR had a higher percentage of follicles >5 mm on D3 compared to sows with high ESR (30% vs. 10%; $P = 0.04$). Thus, sows in which follicle growth was less suppressed during altrenogest feeding had a lower ESR. These effects on follicle development and ESR were more pronounced in split-weaned sows.

Keywords: altrenogest, embryo survival, follicle development, split-weaning, sow

5.1 Introduction

In sows, post weaning altrenogest feeding has been found to sometimes improve ovulation rate (Martinat-Botté et al., 1995; Koutsotheodoros et al., 1998; Patterson et al., 2008), farrowing rate (Martinat-Botté et al., 1990; Morrow et al., 1990), embryo survival rate (Patterson et al., 2008) and litter size (van Leeuwen et al., 2011[a]).

A sow's response to post weaning altrenogest feeding may depend on duration of altrenogest feeding (Patterson et al., 2008; van Leeuwen et al., 2010) or on follicular size at initiation of altrenogest feeding (van Leeuwen et al., 2011[a]). For example, sows with large follicles at weaning had decreased farrowing rates after an 8 days post weaning altrenogest feeding (van Leeuwen et al., 2011[a]), possibly due to "ageing" of the larger follicles during altrenogest feeding. Follicle size at weaning is related to the metabolic impact of lactation on the sow. Alleviating this burden by split-weaning three days prior to weaning resulted in an increase in LH secretion (concentration and pulse frequency) and a higher number of follicles >3 mm on the day after weaning compared to conventionally weaned sows (Zak et al., 2008).

In this study, split-weaning (litter size reduced to the 6 smallest piglets 3 days before weaning) was used in half of the sows, to create differences in follicle size at weaning, to subsequently study changes in follicle size during and after post weaning altrenogest feeding in relation to subsequent reproductive performance. It was hypothesized that split-weaned sows would have larger follicles at weaning, which would lead to reduced reproductive performance after post weaning altrenogest feeding.

5.2 Materials and Methods

5.2.1 Animals, split-weaning, housing and diets

The study was conducted from January 2010 till April 2010 at research facility "De Haar" of Wageningen University and approved by its "Ethical Committee for Animal Experiments". Multiparous sows (n = 47; parity 3-10; Topigs20, Vught, The Netherlands) were randomly assigned to one of two treatments: split-weaning (SW; n = 23), where litters were reduced to the smallest six piglets (smallest of the litter) during the last three days of lactation, or control (C; n = 24), where litter sizes were not reduced. Final weaning took place at 0800 h on day 24.1 ± 3.4 of lactation. Lactation length differed between treatments (22.8 ± 3.3 and 25.5 ± 2.9 d for C and SW, respectively; $P = 0.006$). Average litter size (before SW) was 11.8 ± 2.2 piglets (11.7 ± 2.3 and 12.1 ± 2.2 for C and SW, respectively; $P > 0.1$).

After weaning (weaning = Day 0) sows were transported to the research facility where sows were weighed, back fat depth was measured using A-mode ultrasound (Lean Meater ®, Renco Corporation, Minneapolis, USA) at the P2 spot and sows were moved to individual crates. Sow weight and back fat depth at weaning averaged 251 ± 30 kg and 16.3 ± 3.1 mm, respectively and did not differ between treatments ($P > 0.1$). The ambient temperature was set at 22° C and lights were on between 0700 h and 2300 h.

From weaning till estrus sows were fed twice a day (0800 h and 1600 h) a total of 3.5 kg/day of a commercial lactation feed (Rijnvallei, Wageningen, The Netherlands), formulated to contain 14.3 MJ DE with 155 g/kg crude protein and 7.7 g/kg lysine. From estrus till slaughter sows were fed twice a day (0800 h and 1600 h) a total of 2.5 kg/day of a commercial gestation feed (Rijnvallei, Wageningen, The Netherlands), which was formulated to contain 13.5 MJ DE with 141 g/kg crude protein and 5.8 g/kg lysine.

5.2.2 Altrenogest feeding

All 47 sows were given a 7 day oral altrenogest feeding (20 mg/day, Regumate®, Janssen Animal Health, Beerse, Belgium), administered directly into the sows' mouth, at 1600 h on the day before weaning (Day -1), at 1100 h on Day 0 and at 0900 h on Days 1-5.

5.2.3 Follicle development

From Day 0 till 5 (during altrenogest feeding), on Day 6 (24h after last altrenogest feeding; start of the follicular phase, van Leeuwen et al., 2010; 2011[a]) and on Day 8, sows were scanned trans-rectally with a multi convex transducer (MyLab 30®, Pie Medical, Maastricht, The Netherlands) to assess average follicle size of the five largest follicles present on one ovary. One ovary was scanned each time since it was considered to be representative for the contra lateral one (N.M. Soede; unpublished results). From the first detection of estrus till ovulation sows were scanned every 12 hours (2000 h and 0800 h) until ovulation occurred. Since follicle size can decline during the last hours before ovulation (Soede et al., 1998), pre-ovulatory follicle size was defined as the largest average follicle size during estrus. From a selected number of sows (n = 17; C = 8 and SW = 9), digitized video clips of Days 0, 3 and 6 were subjected to frame-by-frame viewing and were analyzed with a custom-written Matlab (The MathWorks, Natick, Massachusetts, U.S.A.) script to obtain number and diameter of follicles ≥ 2 mm on both ovaries. The largest diameter of each follicle was measured in multiple (³ 2) consecutive frames. Using this method, follicles larger than 2 mm can be reliably assessed (Gonzalez-Añover et al., 2009). These selected sows were evenly distributed over treatment (C and SW) and were representatively distributed over embryo survival classes (low vs. high).

5.2.4 Estrus detection and insemination

Estrus detection was done twice a day (0830 h and 1630 h) starting at Day 7, using back-pressure testing during fence-line contact with a mature and high-libido boar. Sows showing estrus within 10 d after weaning (46 of 47) were inseminated every afternoon of standing estrus (1330 h), until they had ovulated using non-pooled semen containing 2.2×10^9sperm/AI doses from a commercial AI centre.

5.2.5 Slaughter and Dissection Procedures

Sows were slaughtered at the research facility by electrical stunning and exsanguination at 10 or 10.25 days after detected ovulation. Reproductive tracts were recovered and number, weight

and size of corpora lutea on both ovaries was measured. Both uterine horns were flushed at least twice with 30 ml 0.9% NaCl to obtain embryos. The diameter of each embryo and its embryo blast were measured using a stereo microscope. Embryo survival rate was calculated as (total number of viable embryos/ovulation rate) * 100%.

5.2.6 Progesterone analysis

A 10 ml blood sample of each sow was collected on the day of slaughter to analyze plasma progesterone concentrations. Samples were collected in vacutainers that contained EDTA, and put on melting ice before being centrifuged at 3000 x *g* for ten minutes at 4° C. Plasma was stored at -20° C until further analyses. Progesterone was analyzed using solid-phase ^{125}I Radio Immuno Assay (RIA) methods (Coat-A-Count TKPG ®; Diagnostic Products Corporation, Los Angeles, CA, USA). The intra-assay coefficient of variation was 4.7%. Sensitivity was 0.1 ng/mL.

5.2.7 Statistical analyses

One control (C) sow was deleted from all analyses because on the day of slaughter she had over 250 corpora lutea (CL) and 12 follicular cysts. One C sow did not show estrus within 10 days after last altrenogest feeding and was excluded from estrus, CL and embryo parameters. Further, three SW sows had elongated embryos and one C sows had no embryos. Therefore, embryo data of these sows could not be analyzed.

Data were analyzed using SAS (SAS Inst. Inc., Cary, NC, USA). All parameters were checked for normality using the UNIVARIATE procedure of SAS.

Follicle size was analyzed with the GLM procedure of SAS using repeated measures. The GLM model included treatment and parity (Low; parity 3–4; n = 14, Medium; parity 5–6; n = 17 and High; parity ≥ 7; n = 15), sow within treatment x parity, day and treatment*day. Differences in follicle size between treatments were tested against the error term of sow within treatment x parity and differences between day and treatment were tested against the overall error term. The interaction between treatment and parity was not significant and was therefore omitted from the model.

The GLM procedure of SAS was used to analyze effects of treatment (C or SW) on follicle growth during Altrenogest feeding, follicle growth during the last day of Altrenogest feeding, follicle growth during the follicular phase, onset and duration of estrus, ovulation rate and ovarian- and embryo parameters. Models included class variables parity (low, medium or high) and, for embryo development, gestation length (depending on time of slaughter, gestation length was 10 or 10.25 days). Relevant interactions were added to the model and omitted when not significant.

Because lactation length differed between treatments, the effect of lactation length on follicle size and development, onset and duration of estrus, ovulation rate and ovarian- and embryo parameters was tested within treatments, using the GLM procedure of SAS. No effect of lactation length on any of the above mentioned parameters was found.

To analyze relations between follicle size and embryo survival rate within treatments, sows were divided in low ≤ 63% (SW; n = 10 and C; n = 4) and high > 63% (SW; n = 10 and C; n = 17)

embryo survival rate, because this was the median of embryo survival rate in SW sows. The GLM model included embryo survival class (low vs. high), sow within embryo survival class, Day (Day 0 to Day 6), and the interaction between embryo survival class and day. Differences between embryo survival classes were tested against the error term of sow within embryo survival class and day effects as also interactions between day and embryo survival class were tested against the overall error term.

To study relations between follicle development and embryo development (embryo survival and total number of embryos), the increase in follicle size from D0-D6, from D5-D6 and follicle size at the start of the follicular phase (D6) were analyzed as continuous variables using the GLM procedure of SAS. The model included treatment, (increase in) follicle size and their interaction (which was omitted when not significant). To further study relations with the increase in follicle size during the last day of altrenogest feeding, sows were divided in sows with a decrease in follicle size from Days 5 to 6 (≤ 0 mm; n = 27) vs. sows with an increase in follicle size from Day 5 to 6 (> 0 mm; n= 15) and analyzed using the GLM procedure of SAS. The model included class variables treatment, follicle growth class and their interaction. Relations between follicle size during the last day of altrenogest feeding (D5-6), increase in follicle size during the first two days after altrenogest feeding (D6-8) and embryo survival rate were analyzed, calculating the Pearson correlation coefficients, using the CORR procedure of SAS.

Analysis of the follicle pool (> 2mm) on Days 0, 3, 6 and 8 (n = 17) included main effects of treatment (C *vs.* SW), embryo survival class and their interaction. Average follicle diameter of the 5 largest, the 10 largest and of all follicles and number of follicles in specific diameter size classes (2 to 3 mm; 3 to 5 mm; 5 to 7 mm and ≥ 7 mm) were analyzed using the MIXED procedure of SAS. The models included the main effects of treatment, day, embryo survival class and interactions. Sow was included as random variable.

When main effects were significant, least square means were compared by the least significant difference method adjusted for multiple comparisons according to Bonferroni.

The Pearson correlation coefficients between parameters were calculated, using the CORR procedure of SAS. When the correlation coefficient was significant in both treatments and had a similar r-value, then the overall correlation coefficient is shown.

P-values < 0.05 were considered significant and *P*-values between 0.05 and 0.10 were considered to approach significance. Results are expressed as means ± standard deviation unless stated otherwise.

5.3 Results

5.3.1 Follicle development in control and split-weaned sows

Follicle size at weaning was 3.7 ± 0.5 mm on average and did not differ between treatments (3.6 ± 0.6 and 3.7 ± 0.4 mm, for C and SW, respectively; $P = 0.2$; Figure 1). On Day 1, 2 and 8, but not on other days follicle size was smaller in C than in SW sows (3.9 ± 0.8 vs. 4.3 ± 0.5 mm

on Day 1, 4.4 ± 0.5 vs. 4.6 ± 0.5 mm on Day 2, and 5.3 ± 1.2 vs. 6.1 ± 1.6 mm on Day 8; $P \leq 0.05$). At the end of altrenogest feeding, follicle size was 4.9 ± 1.3 mm on average, follicle size during altrenogest feeding increased by 1.3 ± 1.3 mm on average and pre-ovulatory follicle size was 7.3 ± 1.0 mm on average. Neither of these was affected by treatment.

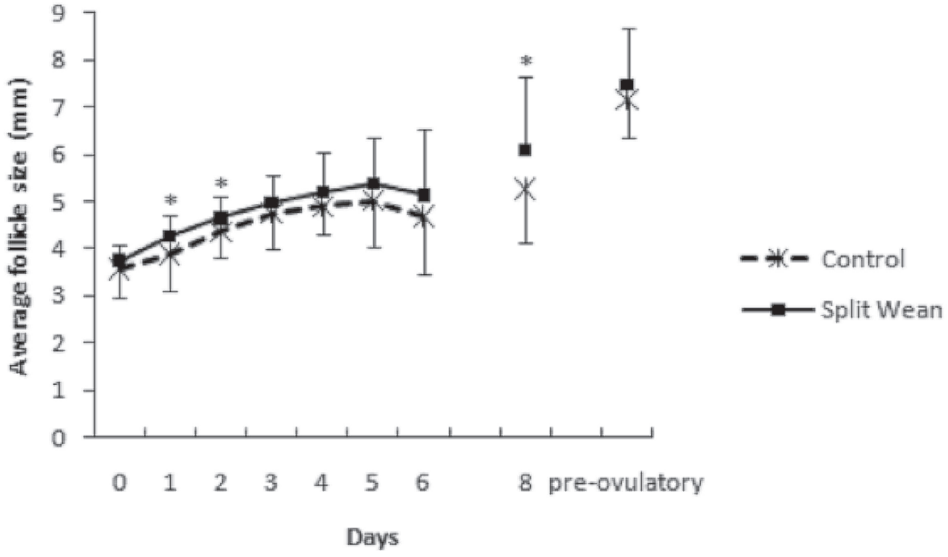

Figure 1. Follicle development (mean ± SD) in control (n = 23) and split-weaned (n = 23) sows during altrenogest feeding and the follicular phase. Day 0 = weaning, Day 6 = start follicular phase and pre-ovulatory = largest average follicular size measured during estrus.
* $P < 0.05$

5.3.2 Estrus, luteal- and embryonic development in control and split-weaned sows

Onset of estrus started 4.3 ±1.0 days after start of the follicular phase and did not differ between treatments (Table 1). No effects of treatment were found on ovulation rate, CL characteristics and progesterone levels. C sows had more embryos (25.1 ± 6.6 and 17.6 ± 9.8 embryos, respectively; $P = 0.005$; Table 1) and a higher embryo survival rate (83 ± 19 and 58 ± 31 %, respectively; $P = 0.001$; Table 1) than SW sows. Average embryo diameter and embryo blast size did not differ between treatments and total number of embryos and embryo survival rate were not affected by parity.

Table 1. Effects of treatment (Control or Split-Weaning) on subsequent reproductive characteristics[a]

	Treatment		
Item	Control	Split-Weaned	*P*-value
No. assigned	23	23	
Onset of estrus (d)	4.4 ±1.0	4.3 ± 1.1	0.84
Duration of estrus (d)	2.8 ± 0.8	2.5 ± 0.7	0.30
Onset follicular phase to ovulation (d)	6.7 ± 0.9	6.7 ± 1.2	0.83
Ovaries			
Ovulation rate (n)[b]	30.4 ± 6.3	30.1 ± 8.3	0.52
Average CL size (mm)	9.9 ± 0.8	9.9 ± 0.9	0.98
Average CL weight (g)	0.41 ± 0.08	0.41 ± 0.09	0.85
Total luteal weight (g)[b]	11.2 ± 3.2	11.5 ± 3.3	0.73
P4 (ng/ml)	42.9 ± 7.2	46.2 ± 10.8	0.23
Embryos[c]	(n = 22)	(n = 20)	
Total number of embryo's	25.1 ± 6.6	17.6 ± 9.8	0.005
Embryo survival rate (%)	83 ± 19	58 ± 31	0.004
Mean embryo size (mm)[d]	6.0 ± 1.9	5.8 ± 2.9	0.47
SD embryo size (mm)[d]	1.58 ± 0.72	1.64 ± 0.82	0.88

[a] Sows were randomly assigned to one of two treatments: split-weaning (litters were reduced to the six smallest piglets three days before weaning) or control, where litter sizes were not reduced until weaning (D0). All sows received a daily oral dosage of 20 mg of Altrenogest from D-1 to D5.

[b] Corrected for parity class $P \leq 0.05$

[c] Data on embryos was not available for four sows due to elongated embryos (all split-weaning) or no embryos (control).

[d] Corrected for gestation length

In split-weaned sows, sows with low (< 63%; n = 10) *vs.* high (> 63%; n = 10) embryo survival rate had a higher follicle size on Days 3, 4, 5, 6 and 8 (Figure 2). In C sows, only 4 of 21 sows had a low embryo survival rate and follicle size did not significantly differ between these embryo survival classes on any of the days.

Overall, embryo survival rate (79 ± 4 vs. 55 ± 6%, LS means, respectively; P = 0.002) and the total number of embryos (23.4 ± 1.6 vs. 17.9 ± 2.1 embryos, LS means, respectively; P = 0.04) were higher in sows that showed a decrease in follicle size from Day 5 to Day 6 than in sows that showed an increase in follicle size from Day 5 to Day 6 (Figure 3). In these analyses, the interaction between treatment and increase in follicle size was not significant. The increase in follicle size from Day 5 to Day 6 was not related to the interval to onset of estrus or to ovulation.

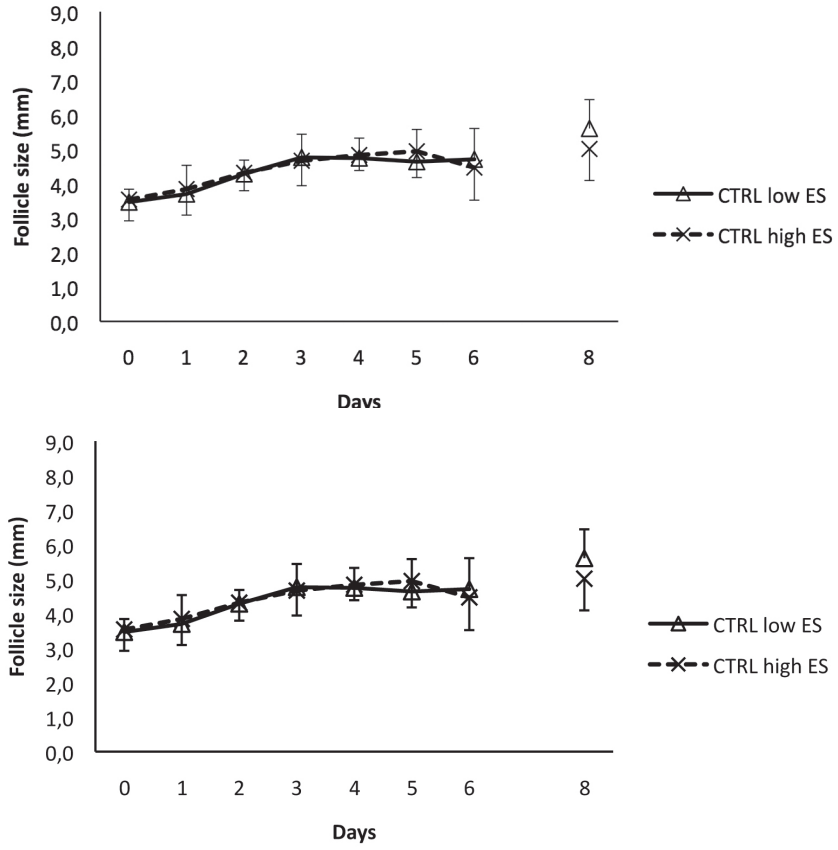

Figure 2. Follicle development (mean ± SD) during altrenogest feeding for (a) split-weaned (SW) sows with low (≤ 63%, n = 10) or high (> 63%, n = 10) embryo survival (ES) and (b) control (CTRL) sows with low (≤ 63%, n = 4) or high (> 63%, n = 17) embryo survival (ES). Day 0 = weaning, Day 6 = start follicular phase. * $P < 0.05$.

5.3.3 Relations between follicle development and reproductive performance

Strong positive relations existed between the increase in follicle size during altrenogest feeding, the increase in follicle size during the last day of altrenogest feeding and follicle size at the start of the follicular phase (r = 0.67-0.93; P < 0.0001). These follicle parameters were all strongly related to embryo survival (similarly in both treatments); every additional mm increase in follicle size during altrenogest feeding, decreased embryo survival rate by 13 ± 3% ($P < 0.0001$); every additional mm increase in follicle size during the last day of altrenogest feeding decreased embryo survival rate by 13 ± 4% ($P = 0.003$) and every additional mm increase in follicle size at the start of the follicular phase, decreased embryo survival rate by 14 ± 3% ($P < 0.0001$). Further, every additional mm increase in follicle size during the last day of altrenogest feeding tended to

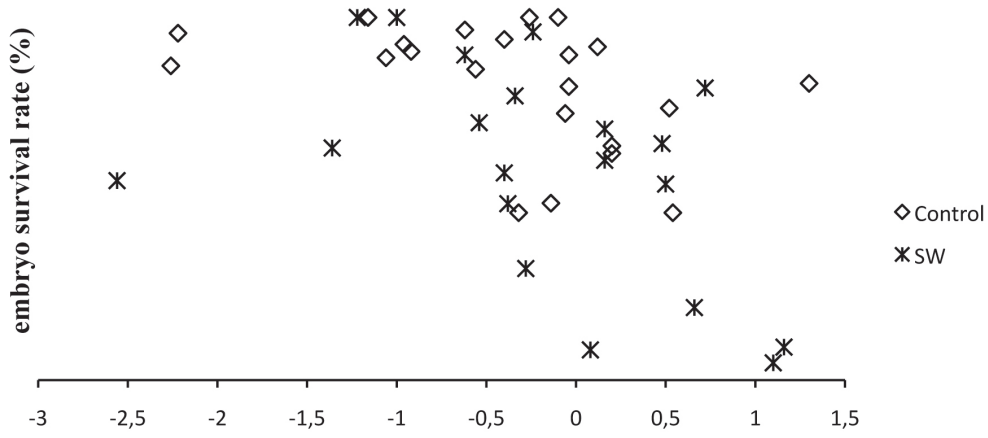

Figure 3. Follicle growth between D5 and D6 (mm) and embryo survival rate on day 10 of pregnancy.

decrease embryo size by 0.8 ± 0.4 mm ($P = 0.06$).

Increase in follicle size during the last day of altrenogest feeding (Day 5-6) was not related to increase in follicle size from Day 6 to Day 8, neither was the increase in follicle size from day 6 to Day 8 related to embryo survival rate or total number of embryos.

5.3.4 Follicle pool

In the 17 sows in which the entire follicle pool (> 2mm) was assessed, average follicle size of the follicle pool was strongly related to average follicle size of the 10 largest follicles and of the 5 largest follicles present, on all days analyzed (10 largest; r = 0.87 (0.80 - 0.90), 5 largest; r = 0.74 (0.55-0.84); $P \leq 0.02$). At weaning, sows had on average 34.3 ± 10 follicles (total no. of follicles; > 2mm), which tended to be higher for Control sows than for Split-weaned sows (37.5 ± 13 and 31.0 ± 6, respectively; $P = 0.06$). Total follicle numbers were similar at Days 3 and 6, but on Day 8 Control sows tended to have more follicles than SW sows (41.6 ± 12 and 31.7 ± 11, respectively; $P = 0.06$). On Day 3, Split-weaned sows with low embryo survival (SW-low) tended to have larger follicles than Split-weaned sows with high embryo survival (4.3 ± 0.5 and 3.6 ± 0.4 mm, respectively; $P = 0.09$), but no differences in follicle size were found on Day 6 or 8.

The distribution of follicles over the different size classes did not differ between treatments on days 0, 3, 6 and 8 (Figure 4), but on Day 3, sows with low embryo survival (≤ 63%) compared with high embryo survival (> 63%) had a higher percentage of sows with follicles > 5 mm (30% vs. 10%; $P = 0.04$), which seemed mainly due to an increase in follicles of 5-7 mm (28% *vs.* 11%; $P = 0.06$) at the expense of follicles of 3-5 mm (42% *vs.* 60%; $P = 0.1$).

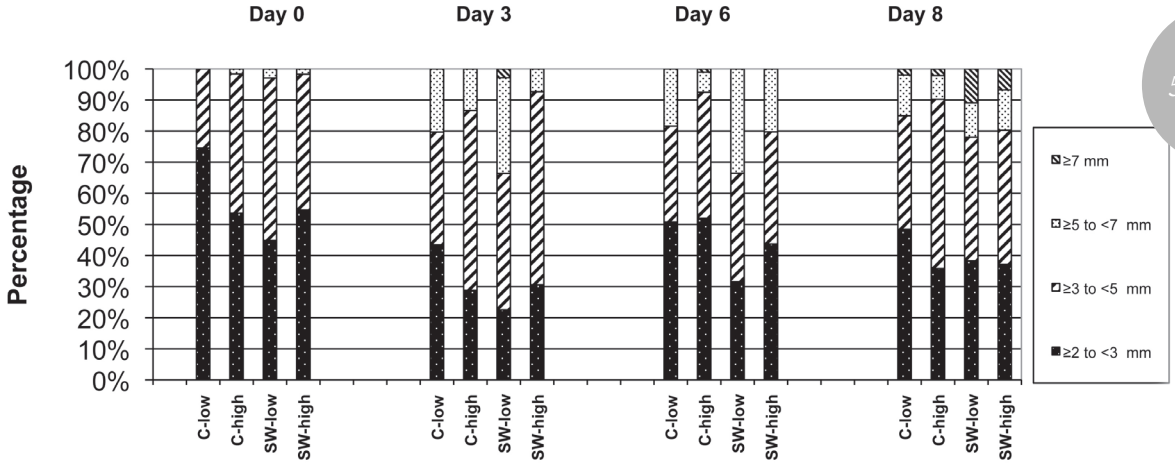

Figure 4. Percentages of follicles in different size classes (class 1; ≥2 to <3 mm, class 2; ≥ 3 to < 5 mm, class 3; ≥5 to < 7 mm and class 4; ≥ 7 mm) in control sows (C) or split-weaned sows (SW) with low ≤ 63% or high > 63% embryo survival (C-low; n = 2, C-high; n = 6, SW-low; n = 5 and SW-high; n = 4).

5.4 Discussion

This study used split-weaning to stimulate follicle development before weaning and study effects on subsequent reproductive performance after post weaning altrenogest feeding, because a previous study showed that a sow's response to post weaning altrenogest feeding is influenced by follicle size at weaning (van Leeuwen et al., 2011[a]). It was expected that split-weaned sows would have larger follicles after weaning because the reduced suckling stimulus after split-weaning causes an immediate increase in LH secretion resulting in more follicles > 3 mm the day after weaning (Zak et al., 2008). Indeed, in our study, at the day after weaning split weaned sows had a higher average follicle size of the 5 largest follicles compared to control sows. No differences were found between split-weaned and control sows for any of the ovarian parameters or P4 levels at slaughter, but split-weaned sows had lower embryo survival rates than control sows (only 58% *vs.* 83%).

Daily assessment of the 5 largest follicles of each sow revealed, that split-weaned sows with low embryo survival had a higher increase in follicle size throughout altrenogest feeding, whereas split-weaned sows with high embryo survival had a lower increase in follicle size, reaching a plateau between Day 3 and 5 and slightly decreasing in size between Day 5 and 6. The differences in average follicle size between SW sows with High or Low embryo survival were significant at Days 3-6 and 8. In control sows, the limited number of sows with low embryo survival (n = 4) made it difficult to make similar comparisons. However, follicle size in control sows remained consistently lower (numerically) than in split-weaned sows, which may similarly affect follicle quality as in the split-weaned sows with high embryo survival rate. Indeed, a decrease in follicle size at the end of altrenogest feeding (between Days 5 and 6) was related to higher embryo survival in both treatment groups. This decrease in follicle size suggests that large follicles became atretic and a new "healthy" set of antral follicles was recruited and selected to ovulate. In the sows with low embryo survival, on the other hand, it seems that the same follicles were persistent and continued growing throughout

the altrenogest feeding period, finally resulting in the selection of aged follicles. This seems to be substantiated with the finding that, at Day 3 after weaning, split-weaned sows with low embryo survival had fewer medium sized follicles (3-5 mm) and higher numbers of larger sized follicles in their follicle pool. These results are in accordance with findings from a previous study (van Leeuwen et al., 2011[a]), in which reduced farrowing rates after a 4-8 day post weaning altrenogest feeding period were found by possible ageing of the larger follicles during altrenogest feeding which could have reduced fertility. It is not clear how exactly ageing may affect embryo mortality. However, it is possible that ageing of follicles ultimately compromises the ovulation process, prohibiting fertilization or results in ovulation of compromised oocytes that are not fertilized or that result in impaired embryo development leading to early embryo mortality. On the other hand, early stages of ageing as we suggest occurs in sows in which follicles decrease in size between Day 5 and 6, may also be an explanation for the higher embryo survival in these sows. Blondin and Sirard (1995) showed in cows that early atresia can have a favorable effect on developmental competence of the oocytes, because early follicular atresia is similar to some intra-follicular changes that happen after the LH surge (Sirard and Blondin, 1996).

The variation in follicle development during altrenogest feeding may be related with variation in (response to) LH-pulsatility. Split-weaning causes an immediate increase in LH levels (Zak et al., 2008), which stimulates follicle growth. Because larger follicles have more LH receptor mRNA (Liu et al., 2000) this allows them to respond more rapidly to LH during the first days of altrenogest feeding. However, individual variation exists in LH-pulsatility after weaning (e.g. van den Brand et al., 2000), which may be related to differences in energy balance (van den Brand et al., 2000) or stress response (Kluivers-Poodt et al., 2010). Sows with a high LH response to split-weaning or final weaning may therefore have a higher stimulation of the larger follicles. Therefore, follicles of sows with larger follicles at weaning or with a larger increase in LH at weaning will probably increase more in size during altrenogest feeding. In the presence of larger follicles, outgrowth of a new cohort of follicles is prevented by the inhibin production of these larger follicles (de Jong, 1988). The prolonged life span of the selected larger follicles can lead to ovulation of an aged oocyte, with decreased maturation and fertilization capacities (Revah and Butler, 1996). Follicle size of the control sows during altrenogest feeding was numerically lower than that of the split-weaned sows, which suggests that indeed split-weaning stimulated follicle development, resulting in increased follicle sizes after weaning. In most control sows, LH levels probably do not increase much during the last days of lactation (Zak et al., 2008), resulting in less developed follicles with less LH receptor mRNA (Liu et al., 2000). These follicles therefore were probably less responsive to LH at weaning and the suppressed LH levels, during altrenogest feeding (Redmer and Day, 1981; van Leeuwen et al. 2011[b]), were not high enough to induce a further increase in follicle size. The fact that embryo survival rate was higher in these control sows may be related to the possibility that such small follicles are less affected by ageing, or that follicle turnover occurred during the altrenogest feeding period.

On the other hand, the reason that some sows show more follicle growth during altrenogest feeding than others, may also be related to the "status" of these follicles at (split-)weaning. Lucy et

al. (2001) showed that synchronized waves of follicle growth exist before weaning and the interval to post weaning estrus may depend on the stage of the cohort of follicles at weaning. Similarly, the status of the follicles at split-weaning or at initiation of altrenogest feeding may determine subsequent responsiveness to LH and therefore follicle growth. In our study, however, follicle development around the end of altrenogest feeding did not affect the interval to ovulation.

Unintentionally, split-weaned sows had longer lactation lengths than control sows (25.5 vs. 22.8 days). Shorter lactation lengths (less than 21 days) have been associated with lower embryo survival rates (see review by Soede et al., 2009), however analyses of lactation length effects within treatments did not reveal any effect of lactation length on embryo survival rate or the number of viable embryos in either of the treatments (results not shown).

In the current study, the entire follicle pool was assessed in 17 animals at 4 moments during altrenogest feeding. A high correlation was found between the average follicle size of the entire follicle pool and the average size of the 10 largest follicles ($r = 0.87$), but also between the average follicle size of the entire follicle pool and the average size of the five largest follicles ($r = 0.71$). Therefore, assessment of the 5 largest follicles is a valid tool to study patterns of follicle development in time. However, obviously, the assessment of only the 5 largest follicles does not allow for assessment of numbers of follicles in different size classes, nor does it provide evidence for recruitment of a new cohort of follicles.

In conclusion, split-weaning stimulated post weaning follicle development, resulting in larger follicles in split-weaned sows compared with control sows. Split-weaned sows had reduced embryo survival rates, especially in SW sows with increased follicle size and sustained follicle growth during altrenogest feeding. It is possible that the prolonged lifespan of these larger follicles resulted in ageing of follicles and oocytes, negatively affecting the chances of embryo survival. On the other hand, the decrease in follicle size from Days 5 to 6 in sows with high embryo survival suggests that slight atresia occurred before ovulation, which can have a positive effect on oocyte competence and thus improved embryo survival.

Acknowledgements

The authors would like to thank Intervet Schering Plough Animal Health and Janssen Animal Health for their financial support and supplying the Regumate and the staff of research facility "De Haar" for care and management of the animals used in the study. We are also grateful to B. Laurenssen and R. Koopmanschap for assistance in the slaughter procedures and the radio immuno assay and a special thanks to dr. M.A. Driancourt for revising the manuscript.

Chapter 6

Variation in LH pulsatility during 24 hours after a postweaning altrenogest treatment in relation to follicle development in primiparous sows

J.J.J. van Leeuwen[1], M.R.T.M. Martens[2], J. Jourquin[3],
M.A. Driancourt[4], B. Kemp[1], N.M. Soede*,

[1]Adaptation Physiology Group, Department of Animal Sciences,
Wageningen University, the Netherlands
[2]Intervet Schering Plough Animal Health, the Netherlands
[3]Janssen Animal Health, Belgium
[4]Intervet Schering Plough Animal Health, R&D Pharmaceuticals, France

Animal Reproduction Science (2011), 126: 101–107

Abstract

This study assessed pulsatile release of LH during altrenogest treatment after weaning in primiparous sows and related this to follicle development, estrus and ovulation rate. Weaned sows (n = 10) received altrenogest 20 mg/day from D-1 to D13 (weaning = D0) at 0800 h. On D13, blood samples were collected every 12 minutes from 1000 until 1900h (1st sampling period) and from 2300h until 0800h (2nd sampling period). During the 1st sampling period, LH concentrations remained low and no LH pulses were detected in 8/10 sows. During the 2nd sampling period, average and basal LH concentrations ($P < 0.04$) and frequency of pulses ($P < 0.0001$) were higher than during the 1st sampling period. Sows with short *versus* long intervals to estrus (< 5 days *vs.* ≥ 5 days) had higher basal and average LH concentrations during the 2nd sampling period ($P \leq 0.004$) and showed more follicular growth during treatment ($P = 0.007$), generating larger follicles at D14 ($P = 0.005$). Sows with high ovulation rate (≥ 25) displayed more LH pulses in total than sows with low (< 25) ovulation rates ($P = 0.03$). In conclusion, this study showed that altrenogest efficiently prevented LH pulsatility during the first sampling period and that low frequency / high amplitude LH pulses were generally present during the second sampling period.
This variability in LH release in between two altrenogest administrations (24h) may explain why follicular growth progresses to 5 mm during altrenogest treatments. LH pulsatility was related to length of the follicular phase and ovulation rate, which signifies its relevance.

Keywords: altrenogest, follicle, lactation, LH, sows

6.1 Introduction

In pigs, altrenogest (a progesterone analogue) treatments are applied to synchronize estrus in gilts and to postpone estrus after weaning in sows to alleviate negative consequences of lactation. Treatment with altrenogest has been reported to have positive effects on fertility, both in gilts (Martinat-Botté et al., 1990) and in sows (increased ovulation rate: e.g. Martinat-Botté et al., 1995, embryo survival rate: Patterson et al., 2008 and litter size: van Leeuwen et al., 2011[a]), although not at all times (sows: Boland, 1983; Everaert et al., 2007; Kirkwood et al., 1986; Martinat-Botté et al., 1985).

Recent studies in our lab (van Leeuwen et al., 2010; 2011[a]; Soede et al., 2007) have shown that follicle size increases during altrenogest treatment, the largest follicles reaching a size of around 5 mm (at dosages of 15-20 mg daily and a treatment length of 4-18 days). This suggests that follicle growth is not fully suppressed during altrenogest treatment. Pulsatile LH release is related to antral follicle development in many studies (Britt et al., 1985; Driancourt et al., 1995). Hence, pulsatile LH release may also support follicular growth during altrenogest treatment. Redmer and Day (1981) found that LH concentrations remained low throughout altrenogest treatment. In their study, however, a low sampling frequency was used, and no further data are available on pulsatile LH release during altrenogest treatment.

The current study, therefore, investigated LH release patterns during the last day of a postweaning altrenogest treatment in primiparous sows and related this to follicle development.

6.2 Materials and Methods

6.2.1 Animals, housing and diets

The study was approved by the "Ethical Committee for Animal Experiments" of Wageningen University and conducted at research facility "The Haar" of Wageningen University.

On d92 (batch 1) or d98 (batch 2) of gestation, gilts (n = 10; Topigs 20, The Netherlands) were fitted with an indwelling jugular vein catheter, that was inserted surgically under full anesthesia (Soede et al., 1997). Subsequently, gilts were housed in individual farrowing pens. After farrowing, sows were weighed (201 ± 12 kg on average) and back fat depth was measured using A-mode ultrasound (Lean Meater, Renco Corporation, Minneapolis, USA) at the P2 spot (6.5 cm from the midline at the level of the last rib), which averaged 19.6 ± 3.2 mm. Cross fostering was performed within 3 days after farrowing to adjust litter sizes to 9-13 piglets. At 0800 h on d 26 ± 2 of lactation (D0) 11.0 ± 1.2 piglets on average were weaned per sow and sows were weighed (184 ± 13 kg on average) also back fat depth was measured (15.8 ± 3.0 mm on average). During lactation, sows lost 17 ± 10 kg on average which was 8 ± 5 % of their body weight at farrowing and 3.9 ± 4.2 mm of back fat depth which was 18 ± 21% of their back fat depth at farrowing.

During the entire experimental period animals were fed twice per day at 0730 h and 1530 h. During gestation sows received 2.5 kg of a commercial gestation feed formulated to contain 13.5

J DE/kg with 14.1% crude protein and 0.6% lysine (Rijnvallei, Wageningen, The Netherlands). From 5 days before expected farrowing until end of estrus sows received a commercial lactation feed, formulated to contain 14.3 MJ DE/ kg with 15.5% crude protein and 0.8% lysine (Rijnvallei, Wageningen, The Netherlands). Sows received 2.5 kg/day before farrowing and from farrowing onwards the ration was gradually increased from 1 kg at farrowing to a maximum of 7 kg at d14 of lactation. From weaning until end of estrus sows received 3.5 kg/day of the lactation feed and after estrus until d10 of gestation sows received 3 kg/day of the gestation feed. Individual drinking nipples provided ad libitum access to water at all times.

Lights were on between 0700h and 2300 h. Barn temperature was maintained at 19°C during the experimental period.

6.2.2 Altrenogest treatment

Sows received 20 mg of altrenogest (Regumate, Janssen Animal Health, Beerse, Belgium) from D-1 to D13 (weaning = D0). Altrenogest was administered directly into the sows' mouth using a dosing gun at 0800 h every morning.

6.2.3 Blood sampling

Blood samples to measure LH pulsatility were taken every 12 min from 1000 until 1900 h and from 2300 h until 0800 h on D13, being the last day of altrenogest treatment. Blood samples were collected in ice-cooled polypropylene tubes containing 100 µL of EDTA solution (144 mg/ mL saline), placed on ice and centrifuged at 2000 x *g* for 10 minutes at 4°C. Plasma was stored at -20°C until analysis.

6.2.4 Follicle development, estrus and ovulation rate

From D-1 until ovulation sows were scanned trans-abdominally daily (MyLab 30, Pie Medical/Esaote, Maastricht, The Netherlands) to assess average follicle size of the five largest follicles present on one ovary. One ovary was scanned each time since it was considered to be representative for the contra lateral one (N.M. Soede; unpublished results). The follicular phase was defined to start at D14, 24 hours after last altrenogest administration (van Leeuwen et al., 2010, 2011[a]).

Estrus detection was done twice a day (0830 h and 1630h) starting at D14, using back-pressure testing during fence-line contact with a mature, high-libido boar. From the first detection of estrus until ovulation sows were scanned every 12 h (0800h and 2000h) until ovulation occurred. Ovulation was presumed to have occurred when previously present pre-ovulatory sized follicles (6-9 mm) had disappeared. Since follicle size can decline during the last hours before ovulation (Soede et al., 1998), pre-ovulatory follicle size was defined as the maximum average follicle size during estrus. The interval to onset of estrus was calculated from D14 (0830h) until first detection of estrus.

On d10 of gestation animals were slaughtered by stunning and exsanguination after which the reproductive tract was removed and ovulation rate assessed.

6.2.5 LH pulsatility

Plasma LH concentrations were analyzed in triplicate, using homologous double-antibody RIA, following the method described by Cosgrove et al. (1991), with the following modifications: 1% BSA was used in the assay buffer, for the precipitation 50 µL cold Saccel (anti sheep/goat, IDS-AA-SAC2, Lucron Bioproducts BV, Gennep, the Netherlands) was used, after mixing and incubation for 1 hour tubes were centrifuged at 6,240 x g for 6 min at 4°C, aspirated and counted. Porcine LH was supplied by the National Hormone & Peptide Program (NHPP, NIDDK, Dr. Parlow, Harbor-UCLA Medical Center, Torrance, CA, USA). The lower limit of detection was 0.012 ng/ml; the intra- and interassay CV were 7.0% (n = 73) and 6.5% (n = 15), respectively.

Definition of LH pulses was based on the definitions described by Langendijk et al., (2007a) and van den Brand et al. (2000) with a few adjustments. A rise in LH concentration was defined as pulse when (Fig. 1): (1) the maximum concentration is reached within two samples from the previous nadir, (2) there were at least two samples between the peak and the subsequent nadir, and (3) the increase was at least four times larger than the coefficient of variation of duplicate pairs of the assay (which was 0.2 ng/ml). The following LH characteristics were defined for the two sampling periods separately: average and basal concentration, the amplitude and frequency of pulses, average pulse maximum, average pulse area, cumulative pulse area and mean LH concentration during the first and last 2 h of sampling. The average LH concentration was the average of all samples. Basal LH concentration was the average of the six lowest values. The amplitude of a LH pulse was the difference between the maximum value and the previous nadir. Pulse area was defined as average of samples belonging to one LH pulse, starting with previous nadir and ending at the subsequent nadir.

6.2.6 Statistical analyses

Data were analyzed using SAS (SAS Inst. Inc., Cary, NC, USA). All parameters were checked for normality using the UNIVARIATE procedure. Differences in LH parameters between the two sampling periods were tested using the GLM procedure of SAS with the following model: $y = \mu + Period_i + Sow_j + e_{ijk}$, where $Period_i$ is the sampling period (first or second sampling period) and Sow_j is the correction for within-sow variation. The sow effect was never significant, but remained in the model. To test differences between the two sampling periods within sows, average concentration of the two sampling periods was compared with a student's t-test using the T-TEST procedure of SAS for paired observations. Within sampling period average concentration during the first 2 hours and last 2 hours of sampling was compared with a student's t-test using the T-TEST procedure of SAS for paired observations.

Pearson correlation coefficients between LH parameters and follicle size, onset to estrus and ovulation rate were calculated, using the CORR procedure of SAS. When the correlation coefficient was significant in both sampling periods and had a similar r-value, then the overall correlation coefficient is shown.

Sows were classified as low (< 25) and high (≥ 25) ovulation rate (average ovulation rate was

24.6 ± 5.4) and differences in follicle development and LH parameters between these groups (corrected for sampling period) were analyzed, using the GLM procedure of SAS. Sows were also classified as having short (< 5 days) or long (≥ 5 days) intervals to estrus (average interval to estrus was 5.0 ± 0.9 days) and differences in follicle growth during altrenogest treatment, follicle size at the start of the follicular phase, follicular growth during the follicular phase and LH pulsatility during first or second sampling period between sows with short or long intervals to estrus were analyzed, using the GLM procedure of SAS.

P-values < 0.05 were considered significant and *P*-values between 0.05 and 0.10 were considered to approach significance. Results are expressed as means ± standard deviation unless stated otherwise.

6.3 Results

6.3.1 Follicle development, estrus and ovulation rate

Follicle size at weaning was 3.1 ± 0.5 mm and during altrenogest treatment follicles grew 1.2 ± 0.8 mm on average, resulting in an average follicle size of 4.3 ± 0.5 mm at the start of the follicular phase. During the follicular phase, follicles grew by 2.9 ± 0.4 mm on average, resulting in an average follicle size of 7.2 ± 0.5 mm before ovulation. Estrus started 5.0 ± 0.9 d (3.5 - 6.5 d) after the start of the follicular phase on average and sows ovulated 6.5 ± 0.7 d (5.5 – 7.5 d) after the start of the follicular phase. Ovulation rate was 24.6 ± 5.4 on average.

6.3.2 LH pulsatility

Large differences in the pattern of LH secretion were observed throughout the day. During the first 11 h after altrenogest feeding, 8 out of 10 sows failed to display LH pulses and the other 2 sows displayed their first LH pulse 9 h and 10 h after altrenogest administration. In contrast, the pattern of LH secretion was clearly different during the second sampling period (Table 1 and Fig. 1) as pulses appeared in all animals during the second sampling period (second *vs.* first sampling period: 3.1 ± 1.0 *vs.* 0.3 ± 0.7 pulses, respectively; $P < 0.0001$, Table 1 and Fig. 1). Average LH concentrations were higher during the second sampling period ($P < 0.0001$). Basal LH concentrations were higher during the second sampling period than during the first sampling period ($P \leq 0.04$; Table 1). Within sampling period, LH levels during first and last 2 sampling hours were similar.

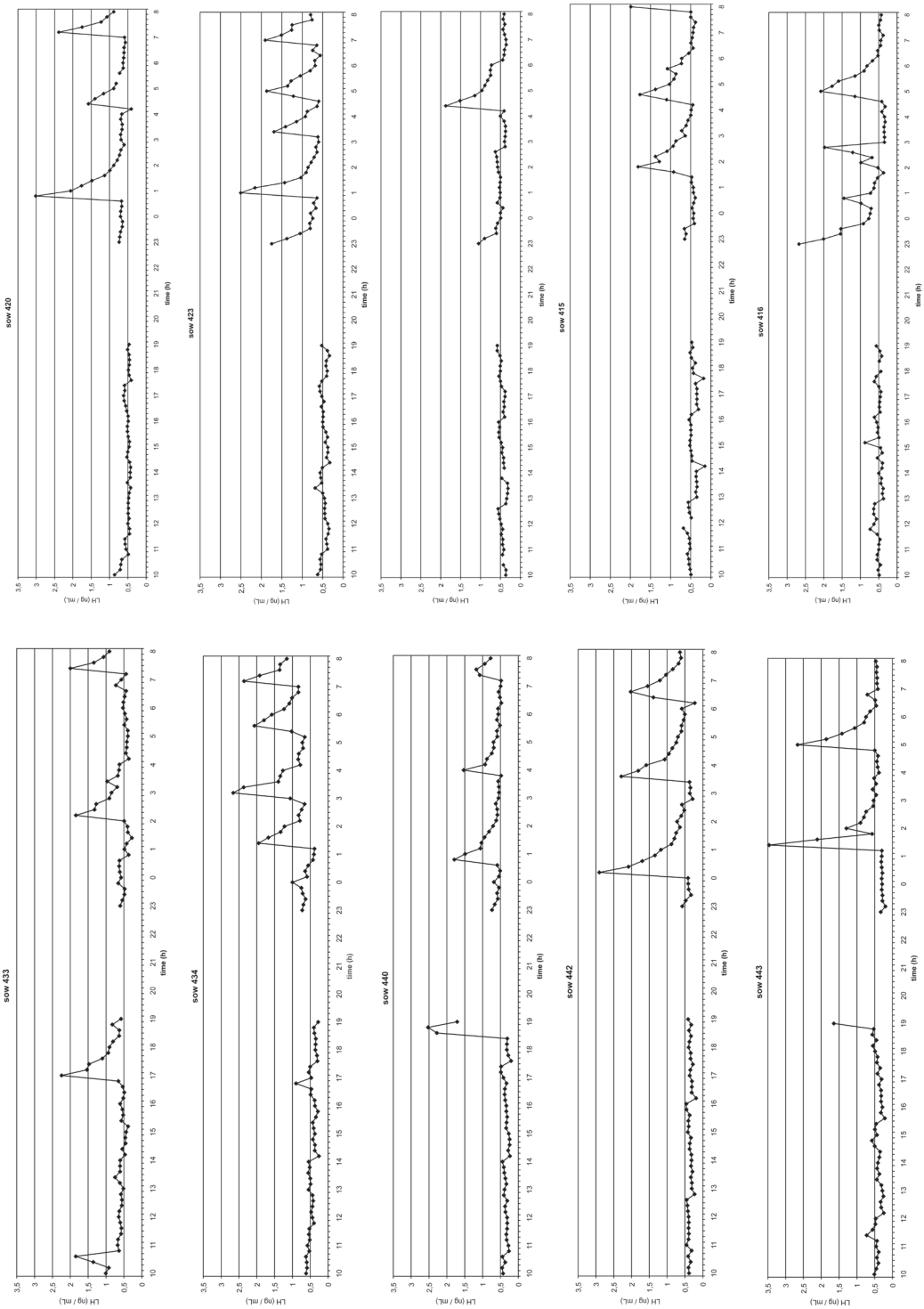

Figure 1. LH concentrations on D13 of 10 sows treated with altrenogest from D-1 till D 13 (weaning = D0). Samples were taken every 12 min during the first (1000 – 1900 h) and second (2300 – 0800 h) sampling period. Altrenogest was fed on D 13 at 0800 h.

Table 1. LH pulsatility during altrenogest treatment (D13) during the first (1000 – 1900h) or second sampling period (2300 – 0800h). Means ± SD.

	D13 (n = 10)		
Parameter	1st sampling period	2nd sampling period	*P*-value
Average LH concentration (ng/mL)	0.49 ± 0.10	0.83 ± 0.17	< 0.0001
Basal LH concentration (ng/mL)	0.33 ± 0.07	0.42 ± 0.11	0.04
No. LH pulses per 9 h	0.3 ± 0.7	3.1 ± 1.0	< 0.0001
Average pulse maximum per 9 h (ng/mL)[a]	2.3 ± 0.3	2.00 ± 0.37	0.35
Average LH amplitude (ng/mL)[a]	1.81 ± 0.6	1.50 ± 0.39	0.36
Average pulse area (ng/mL)[ab]	6.17	8.34 ± 2.34	0.40
Total pulse area (ng/mL)[ab]	6.17	23.9 ± 8.23	0.21
Mean LH concentration first 2 hours (ng/mL)	0.52 ± 0.14	0.78 ± 0.32	0.03
Mean LH concentration last 2 hours (ng/mL)	0.56 ± 0.22	0.78 ± 0.28	0.06

[a] comparison based on only 2 animals with pulses during first sampling period

[b] missing value for one animal during first sampling period due to incomplete profile

Sows with short (< 5 days) compared to long (≥ 5 days) intervals to estrus had larger total LH pulse areas during the second sampling period (31.5 ± 3.9 and 20.6 ± 2.5 ng/mL, LS means, respectively; $P = 0.05$), higher basal LH concentrations during the second sampling period (0.56 ± 0.04 and 0.37 ± 0.03 ng/mL, LS means, respectively; $P = 0.004$) and higher average LH concentrations during the second sampling period (1.0 ± 0.06 and 0.74 ± 0.04 ng/mL, LS means, respectively; $P = 0.003$). Follicles from sows with short intervals to estrus grew more during treatment (1.9 ± 0.3 mm *vs.* 0.9 ± 0.2 mm, LS means, respectively; $P = 0.007$), were larger at the start of the follicular phase (D14: 4.7 ± 0.15 *vs.* 4.1 ± 0.1 mm, LS means, respectively; $P = 0.005$) and grew less during the follicular phase (2.6 ± 0.1 *vs.* 3.1 ± 0.1 mm, LS means, respectively; $P = 0.006$).

Sows with a high ovulation rate (≥ 25; $n = 5$) had more LH pulses than sows with a low (< 25; $n = 5$) ovulation rate (2.2 ± 0.2 and 1.4 ± 0.2 pulses, LS Means, respectively; $P = 0.03$). No differences in follicle growth during treatment, follicle size at the start of the follicular phase and before ovulation were seen between sows with a high or low ovulation rate.

6.3.3 Relations

Follicle size at the start of the follicular phase was related to average LH concentration during the second sampling period ($r = 0.63$; $P = 0.05$; Fig. 2). The increase in follicle size during the follicular phase was negatively related to second sampling period average LH concentrations, total pulse areas and average concentrations during the last 2 hours of sampling ($r = -0.67$; $P = 0.03$, $r = -0.64$; $P = 0.05$ and $r = -0.70$; $P = 0.03$, respectively).

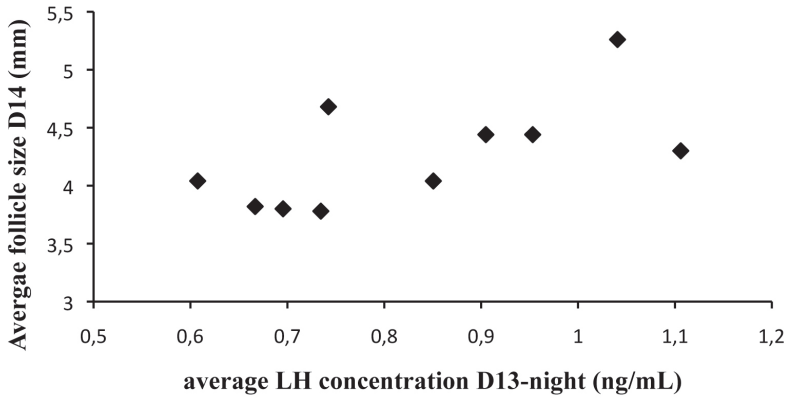

Figure 2. Average LH concentration (ng/mL) on D13 during the second sampling period and average follicle size (mm) on D14 for 10 sows treated with altrenogest from D-1 until D13 (weaning = D 0).

6.4 Discussion

This study investigated LH release during the last day of a postweaning altrenogest treatment in primiparous sows as well as follicle development and subsequent reproductive performance. To our knowledge, this is the first study that investigated pulsatile LH release during altrenogest treatment. Altrenogest binds to the progesterone receptors of target cells in the hypothalamus, suppressing (by negative feedback) GnRH release from the hypothalamus, therefore inhibiting LH and FSH release from the anterior pituitary (Stevenson et al., 1985). When fed at 20 mg/day, peak plasma altrenogest concentrations are reached within 3-6 hours after altrenogest administration (European Medicines Agency; Veterinary Medicines and Inspections, EMEA; 2005). Treatment with altrenogest consistently blocked all pre-ovulatory LH surges and resulted in constant, low LH levels throughout the entire treatment period (Redmer and Day, 1981). According to Redmer and Day (1981), this explains the ability of altrenogest treatment to prevent estrus during treatment and to synchronize resumption of follicular growth following the end of treatment.

This study clearly shows that in 8 out of 10 sows, pulsatile LH release was completely blocked between 2 and 11 h after altrenogest administration. In the last two sows, it was blocked between 3 and 9 h after altrenogest administration. However, during the second, nine hour sampling period, elapsing from 15 to 24 h after altrenogest administration, all 10 sows displayed pulsatile LH release (1-4 pulses reaching around 1.5 ng/ml) A similar low frequency/high amplitude pulsatile release of LH has been shown to occur during progesterone dominance (luteal phase; Dusza et al., 1988; Langendijk et al., 2007[a], d22 of pregnancy; Virolainen et al., 2005 and during late lactation; Langendijk et al., 2007[b]).

The limited suppression of LH release during the second half of the 24 h period follow-

ing altrenogest administration may allow an increase in follicle size up to 5 mm in diameter during altrenogest treatment, regardless of dose or duration of treatment (gilts: Soede et al., 2007 and sows: van Leeuwen et al., 2010, 2011[a]). Hence, LH concentrations observed during altrenogest feeding are adequate to support follicular growth between 3 and 5 mm in diameter, but the low frequency LH release pattern, generated between 14 and 24 h after each altrenogest dose, does not support development of follicles to pre-ovulatory sizes, which depends on high frequency/low amplitude release (Britt et al., 1985; Driancourt et al., 1995).

The impact of the within day variability in the pattern of LH secretion on follicle quality and subsequent fertility is not fully clear. On one hand, a study using an intermittent suckling regime (sows separated from piglets during 12 h per day), demonstrated that LH release was suppressed during the period when piglets were allowed to suckle but not during the separation period (Langendijk et al., 2007[a]), triggering a large variation in onset of estrus in these sows, but not affecting subsequent fertility (Langendijk et al. unpublished results). This would suggest no detrimental effects of a variable LH release pattern over the day on subsequent fertility. On the other hand, in our study, LH concentrations and release patterns during the second sampling period were associated with follicle development, as well as with subsequent interval to estrus and ovulation rate. This seems to indicate that LH release at the end of an altrenogest treatment is related to follicle growth during treatment and subsequent fertility. In previous studies, we found that fertility after an altrenogest treatment was modulated by follicle size at the beginning of treatment and/or follicle development during altrenogest treatment. Farrowing rates were lower (van Leeuwen et al., 2011[a]) and embryo survival rates were reduced (van Leeuwen, unpublished results) in sows that had larger follicles (≥ 4.5 mm compared to < 4.5 mm) at weaning or that showed a larger increase in follicle size during a 6-8 days altrenogest treatment after weaning, respectively. This suggests that growth and aging of the largest follicles may have detrimental effects on subsequent reproductive performance.

Since follicle size at weaning and LH release during altrenogest may vary between animals and/or studies, depending on e.g. the energy balance of sows (Clowes et al., 2003[ab], Zak et al., 1997), this may explain variable effects of altrenogest treatment on subsequent performance (e.g. Boland, 1983; Kirkwood et al., 1986; van Leeuwen et al., 2011[a]; Martinat-Botté et al., 1995; Patterson et al., 2008). Thus, effects of altrenogest treatment after weaning on subsequent performance seem related to the LH release during altrenogest. It may, however, also be (partly) related with direct influences of e.g. insulin and IGF-1 on follicle development (Clowes et al., 2003[b]; Zak et al., 1997). Experiments in which altrenogest treatment would be applied at 12 h intervals could verify the specific role of LH release in subsequent performance.

In conclusion, full suppression of pulsatile LH release was commonly visualized during the first bleeding period but low frequency/high amplitude LH pulses were generally present during the second bleeding period. This variability in the pattern of LH release throughout the 24 h period elapsing between two altrenogest administrations may explain why follicular growth progresses to 5 mm during altrenogest treatments.

Acknowledgements

The authors would like to thank Intervet Schering Plough Animal Health and Janssen Animal Health for their financial support and Janssen Animal Health for supplying the Regumate, the staff of research facility "De Haar" for care and management of the animals used in the study and Dr. George Foxcroft and Shirley Shostak for their help to get the LH assay running. We are also grateful to Bjorge Laurenssen and Rudie Koopmanschap for their contributions to this study and to students and staff from the Adaption Physiology Group of Wageningen University, especially Marlies van 't Hof and Martine Verwoerd, for their help.

Chapter 7

LH, FSH, estradiol profiles and follicle size during a post weaning altrenogest treatment in primiparous sows

J.J.J. van Leeuwen[*], M.R.T.M. Martens[#], J. Jourquin[‡], M.A. Driancourt[§], A. Wagner[≠], B. Kemp[*] and N.M. Soede[*1],

[*]Adaptation Physiology Group, Department of Animal Sciences, Wageningen University, the Netherlands
[#]Intervet Schering Plough Animal Health, the Netherlands
[‡]Janssen Animal Health, Belgium
[§]Intervet Schering Plough Animal Health, France
[≠]University Hohenheim, Institut für Tierhaltung und Tierzüchtung, Germany.

Abstract

This study assessed LH pulsatility before and after weaning, FSH and estradiol profiles, and follicle size, during a 15-day post weaning altrenogest treatment in primiparous sows. Primiparous sows (n = 26) received altrenogest (20 mg) from D-1 to D13 (weaning = D0) at 0800 h. Blood samples were collected every 12 minutes on D-1 and D0 from 0800h till 1700h to assess LH pulsatility and from D-1 till D14 3 times daily (at 0700h, 1500h and 2300h) to asses FSH and estradiol levels. Sows were subjected to ultrasound daily to assess follicle size of the five largest follicles. On D-1 only 3 out of 13 altrenogest sows showed LH pulses compared to 8 out 8 control sows (P = 0.001). On D0, no LH pulses were seen in altrenogest treated sows for the first 4-5 h after weaning/altrenogest administration, but 85% (11 out of 13) of the altrenogest treated sows showed LH pulses hereafter. LH levels increased after weaning, but average LH levels and basal LH levels were lower in altrenogest treated animals than in control animals (average: 0.63 ± 0.16 *vs*. 0.82 ± 0.12 ng/ml, respectively; $P = 0.0009$ and basal: 0.34 ± 0.1 *vs*. 0.47 ± 0.17 ng/ml, respectively; $P = 0.05$). In altrenogest treated sows, both FSH and estradiol levels showed a diurnal release pattern with low levels found 7h after altrenogest administration and high levels 23h after altrenogest administration. Levels of FSH measured on D0 were higher compared to D-1 and subsequently increased till D5 after which FSH levels declined and remained stable. Coinciding with this increase in FSH, follicle size increased till D5 and decreased again. This was thought to be caused by antrum formation of FSH stimulation, since estradiol levels declined after D2, indicating a decreased estrogenic activity of the follicles and thus a disturbed follicular development. In conclusion, altrenogest treatment effectively suppressed pulsatile LH release on the day of weaning during the first 4-5 hours after weaning/ altrenogest administration, but LH pulsatility increased from 5 to 9 hours after treatment. FSH and estradiol levels showed a diurnal release pattern in accordance with time of altrenogest administration. An increase in FSH levels coincided with an increase in follicle size till D5, but follicles showed reduced estrogenic activity after D2 and the increase in follicle size is therefore thought to be the result of antrum formation by FSH instead of normal follicular development, which could explain the reduced fertility after shorter altrenogest treatments as found in previous studies.

Keywords: altrenogest, estradiol, follicle, FSH, LH, sow

7.1 Introduction

Post weaning altrenogest (a progesterone analogue) treatments can be applied in sows to postpone estrus after weaning. Such treatments have been found to improve subsequent reproductive performance parameters in several studies, affecting e.g. ovulation rate (Martinat-Botté et al., 1995), embryo survival rate (Patterson et al., 2008) and litter size (van Leeuwen et al., 2011[a]). Improved performance after altrenogest treatment may be related with the extra recovery time between weaning and subsequent estrus, since also skip-a-heat improves litter size (Clowes et al., 1994; Vesseur et al., 1997). It is also possible that altrenogest treatment itself might affect follicle and/or oocyte quality, since positive effects of altrenogest treatment have been found in gilts (Martinat-Botté et al., 1990; 1995). However, post weaning altrenogest treatments have not always resulted in improved reproduction (Boland, 1983; Everaert et al., 2007; Kirkwood et al., 1986; Martinat-Botté et al., 1985) and have even been found to reduce litter size and farrowing rate (Wergang et al., 2011). These differences in reproductive performance after post weaning altrenogest treatments may be related with differences in follicle size at the onset of and during altrenogest treatment. It has been shown in previous studies that follicle size increases during altrenogest treatment (van Leeuwen et al., 2010, 2011[a], 2011[c]), which raises the question to what extent altrenogest effectively suppresses post weaning LH and/ or FSH levels. Redmer and Day (1981) concluded that LH levels during altrenogest treatment in gilts remain low. However, they did not use frequent blood sampling and could therefore not asses LH pulsatility.

Recently, we established that LH-pulsatility at D13 of a post weaning altrenogest treatment was suppressed for only 9-11h after altrenogest administration and resumed low frequency/high amplitude pulses in the remaining period between 2 altrenogest administrations (van Leeuwen et al., 2011[b]). After weaning, LH release normally follows a high frequency / low amplitude release pattern (e.g. Shaw and Foxcroft, 1985). The question is to what extent the post weaning increase in (pulsatile) LH and FSH release is blocked by altrenogest and how FSH and estradiol profiles, and follicle size develops during altrenogest treatment.

The current study investigated pulsatile LH release at the day before weaning and the day of weaning in altrenogest treated animals and untreated controls and also studied FSH and estradiol levels and follicle size during a 15 day altrenogest treatment.

7.2 Materials and Methods

7.2.1 Experimental set-up in short

Primiparous sows were either given a daily dose of a progesterone analogue (altrenogest, n = 15; 20 mg from D-1 to D13 (D0 = day of weaning)), or were not treated (n = 11). Follicle sizes were monitored daily from d-1 till end of altrenogest treatment, LH-pulsatility was assessed at D-1 and D0, FSH- and estradiol levels were assessed at 8h intervals throughout the treatment period only in altrenogest treated animals.

7.2.2 Animals, housing and diets

The study was approved by the "Ethical Committee for Animal Experiments" of Wageningen University and conducted at research facility "De Haar" of Wageningen University. Pregnant gilts (n = 26; Topigs 20, The Netherlands) arrived at the research facility at the same day, on day 80 (batch 1 (n=12)) and day 60 of gestation (batch 2 (n=14)) and were housed in pairs.

On day 92 (batch 1) or day 98 (batch 2) of gestation an indwelling jugular vein catheter was inserted surgically under full anesthesia according to the procedure described in Soede et al. (1997) and sows were housed in individual farrowing pens.

After farrowing, sows were weighed (on average 201 ± 15 kg) and back fat depth was measured using A-mode ultrasound (Lean Meater, Renco Corporation, Minnaepolis, USA) at the P2 spot (6.5 cm from the midline at the level of the last rib) which averaged 19.2 ± 3.5 mm. Cross fostering was performed within 3 days after farrowing to adjust litter sizes to 9-13 piglets. At 0800h on day 26 ± 2 of lactation on average 11.1 ± 1.2 piglets were weaned per sow. Sows weighed 182 ± 16 kg and had a back fat depth of 15.6 ± 3.6 mm at weaning. After weaning, sows were moved to individual pens with a closed run that was opened after estrus. During lactation, sows lost on average 19.0 ± 9.9 kg, which was 9 ± 5 % of their body weight at farrowing and 3.7 ± 4.7 mm of back fat depth which was 17 ± 24 % of their back fat depth at farrowing.

During the entire experimental period animals were fed twice daily at 0730h and 1530h. During gestation sows received 2.5 kg of a commercial gestation feed formulated to contain 13.5 J DE/kg with 14.1% crude protein and 0.6% lysine (Rijnvallei, Wageningen, The Netherlands). From 5 days before expected farrowing till end of post weaning estrus sows received a commercial lactation feed, formulated to contain 14.3 MJ DE/ kg with 15.5% crude protein and 0.8% lysine (Rijnvallei, Wageningen, The Netherlands). From farrowing onwards the feed ration was gradually increased from 1 kg at farrowing to a maximum of 7 kg at day 14 of lactation. From weaning till end of altrenogest treatment sows received 3.5 kg/day of the lactation feed. Individual drinking nipples provided ad libitum access to water at all times.

Lights were on between 0700h and 2300h. Ambient temperature was maintained at 19 °C till insertion of the catheter, after which ambient temperature was raised to 24 °C and gradually reduced to 19 °C again in 5 days. Three days before expected farrowing of the first sow, temperature was raised to 24 °C and gradually reduced to 19 °C in 5 days after last farrowing.

7.2.3 Altrenogest treatment

Two days before weaning sows were assigned to one of two treatments: control (no treatment; n = 11) or altrenogest (20 mgs of altrenogest from D-1 to D13 at 0800h; weaning = Day 0 at 0800h; n = 15). Sows were assigned to treatments such that farrowing weight, back fat depth and follicle size at D-2 were similar between treatments. Altrenogest (Regumate, Janssen Animal Health, Beerse, Belgium) was administered directly into the sows' mouth. From weaning onwards, sows of the two treatments were housed in different rooms to avoid boar stimulation in altrenogest treated animals before the end of treatment.

7.2.4 Blood sampling

Blood samples (2 mL) to measure LH pulsatility were taken on D-1 and D0 at 12 min intervals from 0800h (weaning time at D0) till 1700h. From D-1 till D13, blood samples (10 mL) were taken from altrenogest treated animals every 8 hours (0700 h, 1500h and 2300h) to determine FSH and estradiol levels.

Blood samples were collected in ice-cooled polypropylene tubes containing 100 µL of EDTA solution (144 mg/mL saline), placed on ice and centrifuged at 2,000 x *g* for 10 minutes at 4°C. Plasma was stored at -20°C until analysis.

7.2.5 Follicle size

From D-1 till ovulation sows were scanned trans-abdominally daily (MyLab 30**,** Pie Medical/ Esaote, Maastricht, The Netherlands) to assess average follicle size of the five largest follicles present on one ovary. One ovary was scanned each time since it was considered to be representative for the contra lateral one (N.M. Soede; unpublished results).

7.2.6 Hormone assays

7.2.6.1 LH assay

Plasma LH levels were analyzed in triplicate, using homologous double-antibody RIA, following the method described by Cosgrove et al. (1991), with the following modifications: 1% BSA was used in the assay buffer, for the precipitation 50 µL cold Saccel (anti sheep/goat, IDS-AA-SAC2, Lucron Bioproducts BV, Gennep, the Netherlands) was used, after mixing and incubation for 1 hour tubes were centrifuged at 6,240 x g for 6 min at 4°C, aspirated and counted. Porcine LH was supplied by the National Hormone & Peptide Program (NHPP, NIDDK, Dr. Parlow, Harbor-UCLA Medical Center, Torrance, CA, USA). The lower limit of detection was 0.012 ng/ml; the intra- and interassay CV were 7.0% (n = 73) and 6.5% (n = 15), respectively.

Definition of LH pulses was based on the definitions described by Langendijk et al., (2007[ab]) with a few adjustments. A rise in LH levels was defined as pulse when: (1) the maximum level is reached within two samples from the previous nadir, (2) there were at least two samples between the peak and the subsequent nadir and (3) the increase was at least three times greater than the coefficient of variation of duplicate pairs of the assay (which was 0.17 ng/ml). The following LH characteristics were defined for the two sampling periods separately: average and basal levels, the amplitude and frequency of pulses, and mean LH levels during the first and last 2 hours of sampling. The average LH level was the overall average of all samples. Basal LH level was the average of the six lowest values. The amplitude of an LH pulse was the difference between the maximum value and the previous nadir (Langendijk et al., 2007[ab]).

7.2.6.2 Estradiol assay

Plasma estradiol levels were assessed in a single RIA in triplicate according to the procedure described in Yang et al. (2000). To ensure that all sample potencies were estimated from the linear part of the standard curve, neat and a 20 fold dilution in PBS gel buffer were carried out. A serially

diluted plasma pool was parallel to the standard curve. Extraction efficiency was 72.35 +/- 0.57 % and estimated potencies were not corrected for recovery. Assay sensitivity, defined as 86 % of total binding, was 0.35 pg/mL. The intra assay CV was 9.6 %. and the inter assay CV was 15.9 %.

7.2.6.3 FSH assay

FSH determinations were performed in duplicate according to the assay procedure described in Wagner et al., (2006) with a standardized double antibody RIA. FSH (AFP 10640B) for standards and iodination and the species-specific antiserum for FSH (AFP 2062096RB) were obtained from Dr. Parlow (NHPP, NIDDK, Dr. Parlow, Harbor-UCLA Medical Center, Torrance, CA, USA). The FSH antiserum was used at a final dilution of 1:200 000. The sensitivity was 36 pg/mL, the intra-assay coefficient of variation was 6 % and the inter-assay coefficient was 7 %. Cross reactivity with LH was less than 0.001%.

7.2.7 Statistical analyses

Of the initial 26 sows, one control sow was excluded from all analyses because she showed estrus at weaning and one altrenogest sow was excluded from all analysis because of a non-functional catheter.

Data were analyzed using SAS (SAS Inst. Inc., Cary, NC, USA). All parameters were checked for normality using the UNIVARIATE procedure of SAS. The GLM procedure of SAS was used to analyze effects of treatment (control or altrenogest) on follicle size. Models included class variables batch (1 or 2). The interaction between treatment and batch was added to the model and omitted when not significant.

Differences in LH characteristics between treatments and sampling day (day before weaning or day of weaning) were analyzed using the GLM procedure of SAS. The model included treatment, sow, day of sampling, batch and the interactions between treatment and day of sampling and between treatment and batch. Interactions were omitted when not significant.

The effect of treatment on the distribution of sows showing LH pulses was tested using the FREQ procedure of SAS with a Fisher's exact test.

To analyze differences in LH levels between treatments within day of sampling the GLM procedure of SAS was used. The GLM-model included treatment, batch (1 or 2), sow within (treatment *batch), time of sampling and the interaction between treatment and time of sampling. Differences in LH levels between treatments were tested against the error term of sow within (treatment*batch) and differences between time of sampling and the interaction between treatment and time of sampling were tested against the overall error term. Differences in LH levels between the first 2 hours of sampling and the last 2 hours of sampling were tested with a Student's T-test for paired observations using the TTEST procedure of SAS.

Differences in FSH or estradiol levels between days and time of sampling were tested using the GLM procedure of SAS. Models included the class variables sow, day of sampling (d-1 till d 14) and time (0700h, 1500h or 2300h). The interaction between day of sampling and time was added to the model and omitted when not significant. Differences in follicle size between days

were tested with a Student's T-test for paired observations using the TTEST procedure of SAS.

In altrenogest treated sows, relations were studied between LH release before and after weaning and FSH release, estradiol release and follicle size during treatment by calculating the Pearson correlation coefficients using the CORR procedure of SAS.

P-values < 0.05 were considered significant and *P*-values between 0.05 and 0.10 were considered to approach significance. Results are expressed as means ± standard deviation unless stated otherwise.

7.3. Results

7.3.1 LH

Pulsatile LH release on D-1 was completely blocked during the nine hours after altrenogest administration in altrenogest treated animals while LH release was seen in control sows. Profiles of altrenogest treated animals on D0 showed an initial suppression of LH release during approximately the first 4.5 hours after which LH pulses were seen and control sows showed pulsatile LH release from shortly after weaning onwards. Figure 1 shows representative LH profiles of 2 altrenogest and 2 control sows on D-1 and D0.

Of the 13 altrenogest treated sows only 3 showed LH pulses during D-1 compared to 8 out of 8 sows in control sows ($P = 0.001$; table 1), which resulted in fewer pulses for altrenogest treated sows than for control sows (0.3 ± 0.7 *vs.* 2.5 ± 1.4 pulses, respectively; $P = 0.001$; table 1). Therefore, average LH levels tended to be lower for altrenogest treated sows than for control sows (0.46 ± 0.08 *vs.* 0.56 ± 0.14 ng/ml, respectively; $P = 0.06$; table 1). This difference was mainly seen from 1000-1500h ($P = 0.04$; table 1; figure 2) and from 1500-1700h ($P = 0.02$; table 1; figure 2).

On D0, pulsatile LH release was completely suppressed in altrenogest treated sows during on average the 4.5 h following weaning (0800h), after which LH pulses were seen in 85% of the altrenogest treated sows (11 out of 13; table 1). Control sows, showed LH pulses from on average 1.5 h following weaning onwards and pulses were seen in 100% of the control sows (8 out of 8; table 1). This resulted in 2.7 ± 2.2 *vs.* 3.1 ± 2.0 pulses for altrenogest treated and control sows, respectively ($P > 0.1$; table 1).

On D0 no differences in LH levels were seen during the first 2 hours after weaning/altrenogest administration between altrenogest treated and control animals, but LH levels were lower for altrenogest treated animals between 1000h and 1500h (0.55 ± 0.16 *vs.* 0.84 ± 0.15 ng/mL, respectively ($P = 0.0005$; table 1, figure 2). No difference in LH levels was seen during the last 2 hours of sampling ($P > 0.1$; table 1, figure 2).

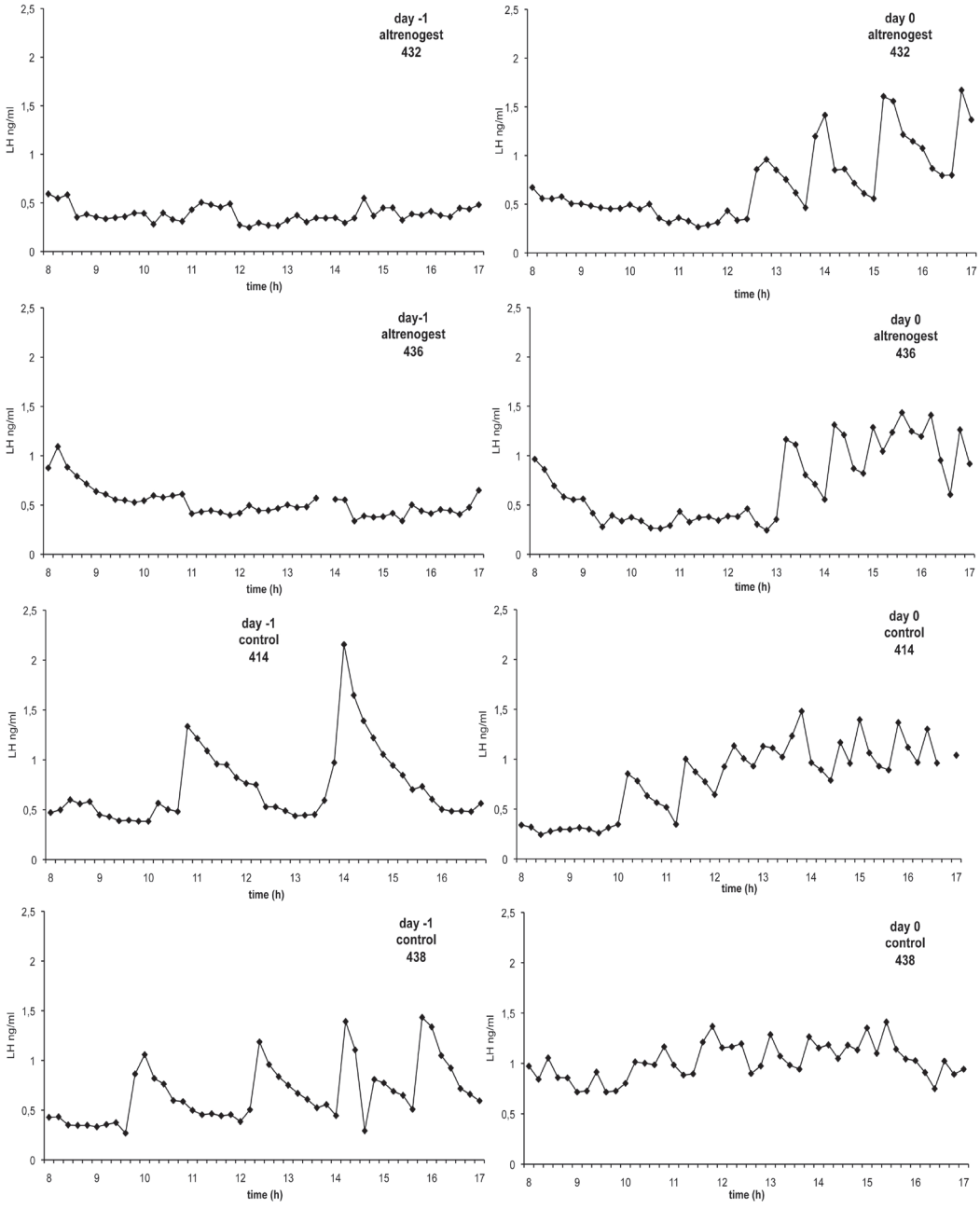

Figure 1. LH levels (ng/mL) on the day before weaning (D-1) and the day of weaning (D0) for 2 representative altrenogest-treated sows (a) and 2 representative control sows (b). Altrenogest was supplied to altrenogest-treated sows at 0800 h.

Table 1. LH levels and pulsatility (mean ± SD) for Control and Altrenogest treated primiparous sows at D-1 and D0[1]

	D-1			D0		
Parameter	Control (n = 8)	Altrenogest (n=13)	P	Control (n = 8)	Altrenogest (n = 13)	P
Mean LH level (ng/ mL)	0.56 ± 0.14	0.46 ± 0.08	0.06	0.82 ± 0.12	0.63 ± 0.16	0.0009
Basal LH level (ng/ mL)	0.32 ± 0.08	0.32 ± 0.07	0.96	0.46 ± 0.17	0.34 ± 0.10	0.05
Mean LH 0800-1000h (ng/mL)	0.52 ± 0.18	0.54 ± 0.12	0.78	0.59 ± 0.22	0.59 ± 0.15	0.94
Mean LH 1000- 1500h (ng/mL)	0.56 ± 0.16	0.44 ± 0.08	0.04	0.84 ± 0.15	0.55 ± 0.16	0.0005
Mean LH 1500- 1700h (ng/ mL)	0.61 ± 0.19	0.44 ± 0.10	0.02	0.93 ± 0.13	0.82 ± 0.28	0.32
Sows with LH pulses	8/8	3/13	0.001	8/8	11/13	0.5
No. LH pulses per 9 h	2.5 ± 1.4	0.3 ± 0.7	0.001	3.1 ± 2.0	2.7 ± 2.2	0.64

[1] Altrenogest treatment was administered daily at 0800h on D-1 and D0. Weaning (= D0) took place at 0800h and sows were sampled every 12 minutes on D-1 and D0 from 0800h till 1700h

In both altrenogest treated animals and control animals an increase in LH levels was seen during the sampling period (figure 2). LH levels were higher during the last 2 hours of sampling, than during the first 2 hours of sampling in both treatments (control; $P = 0.007$, altrenogest; $P = 0.03$), but average LH levels and basal LH levels were lower in altrenogest treated animals than in control animals (0.63 ± 0.16 *vs.* 0.82 ± 0.12 ng/mL, respectively; $P = 0.0009$ for average LH levels and 0.34 ± 0.1 *vs.* 0.47 ± 0.17 ng/mL, respectively; $P = 0.05$ for basal LH levels; table 1).

7.3.2 FSH during altrenogest treatment

During altrenogest treatment FSH levels varied (figure 3). On D-1 FSH levels showed a decline from 0700 to 1500h, after which FSH levels increased again and on D0 FSH levels increased from 0700 to 1500h and from 1500 to 2300h (figure 3). A clear daily pattern was seen in FSH

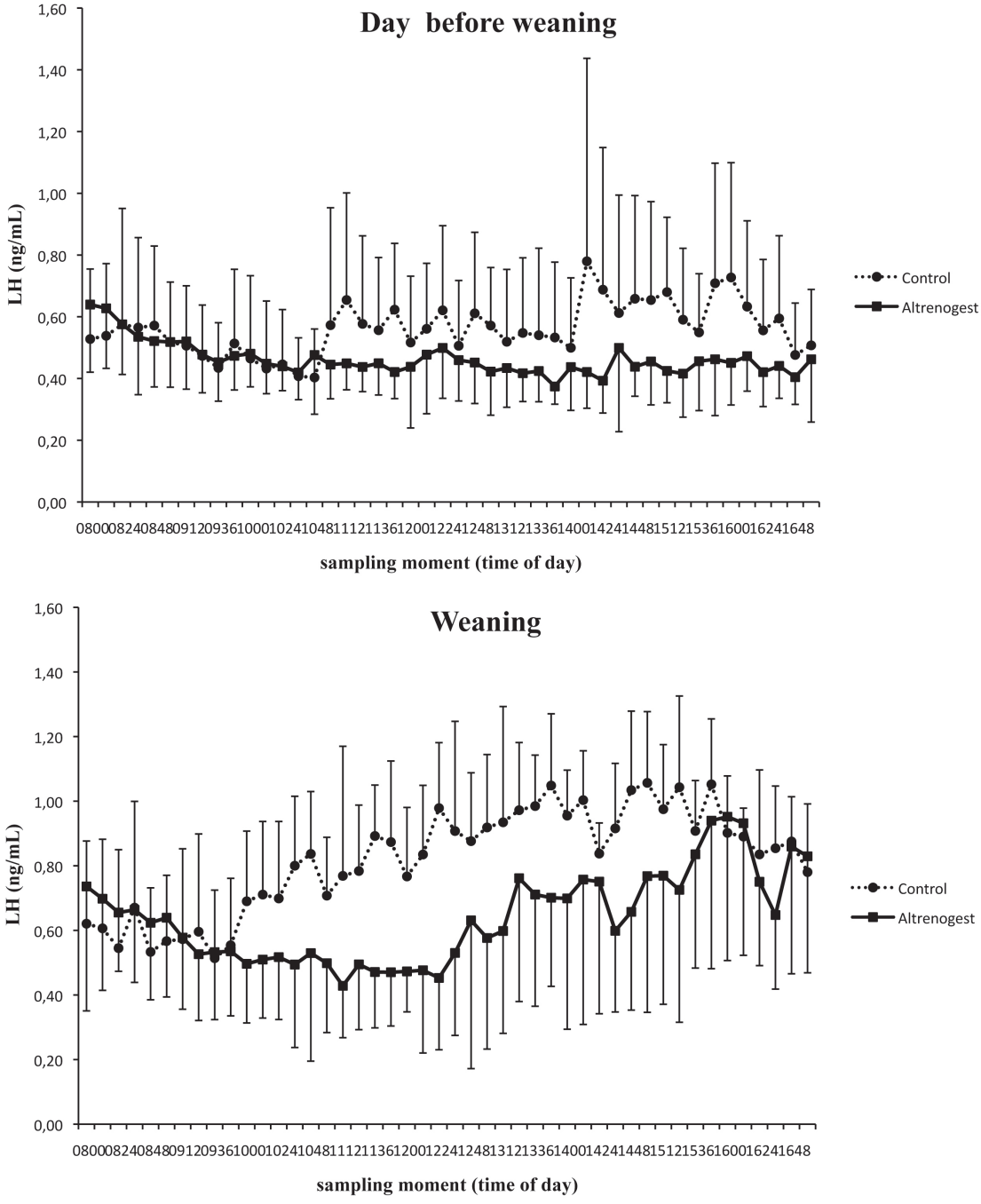

Figure 2. Average LH levels (ng/mL) on the day before weaning and the day of weaning (=D0) for control and altrenogest-treated sows (mean ± SD).

release throughout altrenogest treatment with highest levels of FSH normally found at 0700h (23 hours after last altrenogest administration) and lowest levels normally found at 1500h (7 hours after last altrenogest administration) resulting in different FSH levels for the three daily sampling moments (1.93 ± 0.03 *vs.* 1.62 ± 0.03 *vs.* 1.83 ± 0.03 ng/ml, LS Means, for 0700h, 1500h and 2300h, respectively; $P < 0.0001$; figure 3).

Daily FSH levels increased till D5 after which FSH levels declined and remained stable hereafter. This resulted in different FSH levels between days ($P < 0.0001$; figure 3). Average LH levels on D0 were related to FSH levels on 0 ($r = 0.61$; $P < 0.0001$)

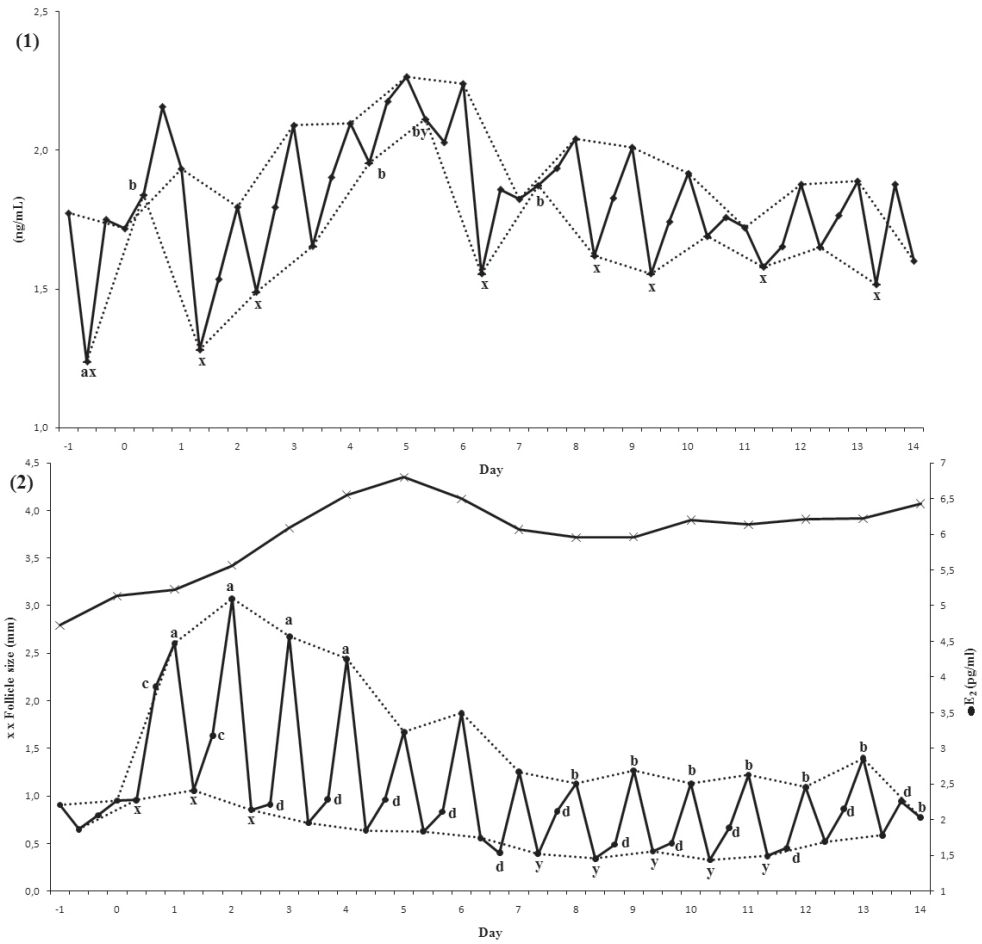

Figure 3. Average FSH levels (ng/mL)(1), follicle size (mm) and estradiol (E_2) levels (pg/mL) (2) during Altrenogest treatment. Altrenogest was administered every morning at 0800 h from D-1 till D13 and blood samples were taken every 8 hours at 0700 h, 1500 h and 2300 h. ab, xy, cd; $P \leq 0.05$

7.3.3 Follicle development; size and estradiol levels

Follicle size at weaning was on average 3.1 ± 0.4 mm and did not differ between altrenogest treated and control sows. During altrenogest treatment follicle size increased from 3.1 ± 0.5 mm at weaning to 4.4 ± 0.6 mm on D5, after which follicle size declined again to 3.7 ± 0.5 mm on D9 and remained stable (figure 3). Follicle size after treatment (D14) was 3.9 ± 0.5 mm which was higher than at weaning ($P = 0.02$). Average LH levels on D0 were related to average follicle size on D0 ($r = 0.37$; $P = 0.0002$).

Estradiol levels also varied during altrenogest treatment (figure 3). On D-1 estradiol levels decreased slightly from 0700h to 1500h and increased thereafter till 0700h on D1 (figure 3). A clear daily pattern was seen in estradiol release throughout altrenogest treatment with highest levels of estradiol normally found at 0700h (23 hours after last altrenogest administration) and lowest levels normally found at 1500h (7 hours after last altrenogest administration), but the difference between sampling moments became smaller as altrenogest treatment progressed (P (interaction day * sampling moment) < 0.0001; figure 3).

Average estradiol levels increased till D2 after which levels declined again and remained stable from D7 onwards (figure 3). Average LH levels on D0 were related to estradiol levels on D0 ($r = 0.40$; $P < 0.0001$).

7.4 Discussion

This study investigated pulsatile LH release around weaning in altrenogest treated primiparous sows and untreated controls and measured FSH and estradiol levels during altrenogest treatment to relate this to follicle size of the five largest follicles during and after treatment.

The day before weaning almost no LH pulsatility was observed in altrenogest treated sows during the nine hours following administration of treatment. Only 3 out of 13 altrenogest sows showed LH pulses while in control sows all sows (8 out of 8) showed LH pulses. LH release in control sows showed the typical low frequency/high amplitude release as is known to occur during lactation (Shaw and Foxcroft, 1985), when serum gonadotrophin levels are low due to the inhibitive effect of the suckling stimulus (DeRensis et al., 1993). Weaning of the piglets results in increased GnRH release and therefore an increase in LH pulsatility after weaning, which shows a typical high frequency/low amplitude release pattern (Shaw and Foxcroft, 1985; Quesnel and Prunier, 1995).

Pulsatile LH release on D0 in altrenogest treated animals was almost completely suppressed during the 4.5 hours following weaning/altrenogest, indicating that altrenogest indeed provides a negative feedback to the hypothalamus, slowing GnRH release and so inhibiting LH release from the anterior pituitary (Stevenson et al., 1985), during the first hours after altrenogest administration. During the second half of the sampling period (5-9h after altrenogest administration) LH pulses were already seen in 11 out of 13 altrenogest treated sows, which indicates that LH release was not suppressed for a long period of time after altrenogest administration. This is in contrast with the assumption of Redmer and Day (1981), that LH levels remain low during altrenogest treatment. They found in gilts

that altrenogest effectively suppressed LH release throughout treatment. The findings of this study are consistent with the results from a previous study, where LH release was studied on the last day (d13) of a 15 day post weaning altrenogest treatment (van Leeuwen et al., 2011[b]). In that study also pulsatile LH release was seen in 10 out of 10 sows during the 15 to 24 hours following altrenogest administration. During the first 11 hours after altrenogest administration, however, only 2 out of 10 sows showed pulses indicating that LH pulsatility was more suppressed on day 13 than on the day of weaning. This indicates that LH is less suppressed by altrenogest on the day of weaning. This may be caused by the enhanced metabolism of sows coming out of lactation (Clowes et al., 1998), which has probably increased altrenogest's clearance rate and therefore reduced its effectiveness. Also the increased LH pulsatility induced by weaning (Shaw and Foxcroft, 1985) may have contributed to this effect.

Levels of FSH and estradiol showed a clear diurnal pattern with low levels found 7h after altrenogest administration and high levels 23h after altrenogest administration. LH is only blocked during first hours after altrenogest treatment, which is likely to be the result of blocking GnRH pulses. With restoration of GnRH pulses and especially when GnRH is released in a low frequency, as is the case during altrenogest treatment, also FSH may increase (Dalkin et al., 1989; Burger et al., 2002). This could explain the diurnal pattern in LH and FSH release from the pituitary, and the diurnal pattern in E2 secretion by these relatively small follicles. An increase in FSH levels was seen from D1 till D5 after which FSH levels decreased again. Coinciding with increasing levels of FSH, follicle size increased during treatment and maximum follicle size was reached at D5 after which follicle size declined again. This is consistent with an earlier study (van Leeuwen et al., 2010), where a plateau in follicle growth during altrenogest treatment was found around day 6 of treatment and other studies where follicle growth was also seen during altrenogest treatment (van Leeuwen et al., 2011[a]; 2011[c]). Follicle size at day 5 of altrenogest treatment was approximately 4 mm and follicles are known to reach their full LH dependency between 2-4 mm (Liu et al., 2000). This indicates that FSH and LH levels during the first days of altrenogest treatment were sufficient to initiate and sustain follicle growth, but the LH levels during the remainder of the treatment were insufficient to sustain outgrowth of the follicles to pre-ovulatory sizes. Follicular development during progestagen treatments in cattle also shows an increase in size at the beginning of treatment, after which a plateau in follicle size is reached (dairy: Burke et al., 1994 and beef: Bo et al., 1995) and also a higher incidence of follicular atresia occurs during progestagen treatment (Savio et al., 1993). In our study follicles showed estrogenic activity during the first days of treatment because estradiol levels increased from D1 till D2.

After D2 estradiol levels declined and remained lower for the remainder of the treatment. This indicates that follicles lost competence to produce estradiol or were insufficiently stimulated by LH and FSH after some days of treatment. Follicle size however, increased till D5, which seems in disagreement with the reducing estradiol levels. Right after weaning (D0 and D1), the high metabolic status of the sows (Clowes et al., 1998) probably results in a less suppressive effect of altrenogest on LH and FSH release, which explains the initial follicle growth and increase in estradiol levels. As LH levels become more suppressed, when the suppressive effect of altrenogest becomes more apparent, less LH, which is an important stimulant for follicular estradiol production (Cardenas and

Pope, 2002), is available and therefore estradiol levels decline. The decreased estradiol levels were most likely accompanied by a decrease in inhibin production, since inhibin production is lower in atretic follicles (Guthrie et al., 1997), which together allowed for an increase in FSH levels between D2 and D5. FSH is responsible for antrum formation of the follicle (Itoh et al., 2002; Serafim et al., 2010) and it may also cause an increase in size of the follicles by expansion of the antrum. This could explain why follicles increased in size, while follicles were not undergoing normal follicle development, since estradiol concentrations remained low. The question is why follicle size decreased after D5. This decrease in size may indicate that low estrogenic follicles turnover after D5 and a new cohort of follicles is selected. This new cohort does not grow out to similar size as the previous cohort, because LH release is more suppressed later on during altrenogest treatment (van Leeuwen et al., 2011[b]).

A previous study (van Leeuwen et al., 2011[a]) found reduced fertility after an 8 day altrenogest treatment. This was mainly caused by sows with large follicles at weaning and it was hypothesized that this could be caused by aging of the follicles during treatment. The current results indicate that even though follicles increase in size during altrenogest treatment, they show little estrogenic activity, indicating loss of competence, possibly as a result of aging of the follicle. This reduced competence may affect oocyte maturation (Ding and Foxcroft, 1994), so ovulation of these follicles could indeed explain the reduced fertility. This could be an explanation for reduced fertility found after altrenogest treatment in other studies (Dos Santos et al., 2004; Werlang et al., *in press,* Chapter 5).

In conclusion, Altrenogest treatment effectively suppressed pulsatile LH release during the first 4-5 hours after administration of the treatment, but LH pulsatility increased from 5 to 9 hours after treatment. Both FSH and estradiol levels showed a diurnal release pattern related to time of altrenogest administration. FSH levels increased during the first 5 days of treatment and this coincided with an increase in follicle size. This increase in follicle size seems mainly caused by atrum expansion through FSH stimulation, because follicles showed decreased estrogenic activity after 2 days of treatment. This disturbed follicle development may also affect oocyte maturation and therefore explain reduced fertility after altrenogest as found in some other studies.

Acknowledgements

The authors would like to thank Intervet Schering Plough Animal Health and Janssen Animal Health for their financial support and Janssen Animal Health for supplying the Regumate, dr. George Foxcroft and Shirley Shostak for their help to get the LH and E_2 assay running and for providing the LH antibodies, dr. Parlow for providing the porcine LH and the staff of research facility "De Haar" for care and management of the animals used in the study. We are also grateful to Rudie Koopmanschap for his help in the catheterization, blood sampling, slaughter procedures and blood analyses, to Bjorge Laurenssen for supervising all experimental procedures and his efforts in catheterization, blood sampling and slaughter procedures and to all students and staff from the Adaption Physiology group, who participated in this study, especially Marlies van 't Hof and Martine Verwoerd.

Chapter 8

General Discussion

8.1 Introduction

Reproductive performance after post weaning altrenogest treatments can be highly variable. This thesis evaluated reproductive performance after different altrenogest treatments and studied physiological events both on follicular and endocrine level. This chapter combines the results of experiments using altrenogest in weaned sows and discusses reproductive results based on the acquired knowledge. In paragraph 8.2, follicle development and endocrine dynamics during altrenogest treatment are discussed. Then, in paragraph 8.3, the effect of application strategy on reproductive outcome is dealt with, after which an evaluation of reproductive performance in studies using post weaning altrenogest treatment is made. Sow related factors that may influence reproductive outcome after altrenogest treatments are discussed in paragraph 8.4. Consequences for the practical application of post weaning altrenogest treatment are given in paragraph 8.5 and recommendations for further research are made in paragraph 8.6.

8.2 Follicular and endocrine dynamics during altrenogest treatment

In all 5 experiments that evaluated follicle development during altrenogest treatment (Chapter 2-5 and 7) an increase in average follicle size (5 largest follicles) was seen during post weaning altrenogest treatment, from 2.5 - 4.2 mm at weaning to 3.8 – 5.6 mm after 7 days of treatment (Table 1). Chapters 2, and 7 evaluated follicle size daily during altrenogest treatment and both revealed a strong increase in follicle size during the first 5-6 days of treatment (e.g. +2.1 mm to an average size of 4.6 mm on Day 6 in Chapter 2 and +1.3 mm to an average size of 4.4 mm on Day 5 in Chapter 7), after which follicle size remained relatively stable till the end of treatment (Day 14; 4.9 mm in Chapter 2 and 3.9 mm in Chapter 7). There is, however, a large variation in follicle size between experiments; 2.5 – 4.2 mm at weaning, 3.8 – 5.6 mm after 7 days of treatment and 3.9 – 5.1 mm after 14 days of treatment (Table 1) and the differences in increase in follicle size during altrenogest treatment (Table 1) do not seem to be affected by follicle size at weaning.

An increase in follicle size was also seen during altrenogest treatment in gilts (Soede et al., 2007) and the authors suggested that altrenogest had a less suppressive effect on LH release than endogenous progesterone, which resulted in some LH release that had stimulated follicle growth. Indeed, the current thesis shows (Chapter 6 and 7) that, although altrenogest (when given once daily) suppresses LH pulsatility during the first hours after treatment, the suppressive effect does not last for 24h. On the day of weaning, LH pulses were found in 11 out of 13 altrenogest treated sows from 4.5 (3.5 - 8) hours after altrenogest administration onwards (Chapter 7) and no pulses were seen within 9 hours after altrenogest administration in the remaining 2 sows. When pulses had resumed, pulse frequency during altrenogest treatment at the day of weaning was not lower than that of untreated control sows (2.7 *vs.* 3.1 pulses in the whole 9 hour sampling period), and also LH levels were similar from 7-9h after altrenogest administration. On Day 13 of treatment LH pulses were suppressed for more hours after treatment; first LH pulses were seen from 9h

Table 1. Follicle size in mm (mean ± SD and range) for Chapter 2-5 and 7 for altrenogest treated sows

	Chapter [1]					
	2	3	4	4	5	7
Parity	1	1	1	2-3	3-10	1
No. assigned	31	165	50	91	46	14
Weaning	2.5 ± 1.2	3.7 ± 0.9	3.9 ± 0.7	4.2 ± 0.9	3.7 ± 0.5	3.1 ± 0.5
After 7 days of treatment	4.6 ± 1.5	5.3 ± 1.4	5.0 ± 0.9	5.6 ± 1.2	4.9 ± 1.3[2]	3.8 ± 0.8
After 14 days of treatment	4.9 ± 0.9	5.1 ± 1.2	-	-	-	3.9 ± 0.5
Follicle growth 0-7	2.1 ± 1.5	1.6 ± 1.7	0.9 ± 1.0	1.8 ± 2.0	-	0.7 ± 0.9
Follicle growth 0-14	2.9 ± 1.2	1.7 ± 2.0	-	-	-	0.8 ± 0.8
Day 4 of the follicular phase[3]	7.8 ± 1.6	7.4 ± 1.3	7.3 ± 0.9	8.5 ± 1.9	7.0 ± 1.1	5.6 ± 0.4

[1] All post weaning altrenogest treatments started the day before weaning and consisted of 8 days 15 or 20 mg or 15 days 15 mg (Chapter 2), 4, 8 or 15 days 20 mg (Chapter 3), 8 days 20 mg (Chapter 4), 6 days 20 mg (Chapter 5) and 15 days 20 mg (Chapter 7).
[2] After 5 days of treatment

after altrenogest administration onwards (Chapter 6). The pulses had a higher amplitude and longer frequency than at the day of weaning, similar to LH profiles under progesterone dominance (e.g. 3.7 pulses/9h; Langendijk et al. 2007[a]). These differences in LH suppression at Day 13 compared to the day of weaning may be related to differences in the clearance rate of altrenogest. As time after weaning progresses, sow metabolism, which was enhanced during lactation, due to the high feeding level and increased metabolism for milk production (Clowes et al., 1998), stabilizes at a lower level. Miller et al. (1999) showed that a high feeding level increases the clearance rate of progesterone, so altrenogest clearance may also be enhanced shortly after lactation, due to the higher metabolic rate during lactation. When sow metabolic rate decreases, altrenogest clearance may also decrease, which would explain the increased period of LH suppression after altrenogest administration on Day 13 of treatment. These results show that during a substantial time of the day LH release is not suppressed by a once-daily-dose altrenogest treatment, which may explain why follicles increase in size during treatment.

Like LH, also FSH and estradiol levels were affected by the suppressive effect of altrenogest on GnRH release. Both FSH and estradiol levels showed a daily release pattern, which was associated with time after altrenogest administration. Blood samples taken at 7 hours, 15 hours and 23 hours after daily treatments, revealed that lowest levels of both FSH an estradiol were found 7 hours after altrenogest administration while highest levels were found after 23 hours

after altrenogest administration. Peak values of FSH were reached on Day 1, probably as a result of increased GnRH release after weaning. FSH levels were lowest at Day 2, which coincided with increased estradiol levels at Day 2. The increase in estradiol levels at Day 2 probably reflected the increase in follicle development as a response to increased LH and FSH levels after weaning. However, as estradiol levels increased, this subsequently resulted in a decrease in FSH levels. When estradiol levels declined after Day 2, possibly also inhibin levels declined, resulting in increased FSH levels up to Day 5. The increase in FSH during the first 5 days of treatment coincided with an increase in follicle size. So, it seems that LH and FSH levels were high enough to stimulate an initial recruitment of follicles from the follicle pool present at weaning. After Day 5, FSH levels decreased. Like for LH, this may be related to reduced clearance of altrenogest, and thus a more suppressive effect of altrenogest on FSH release, when sow metabolism stabilizes at a lower level after weaning. During the follicular phase a positive relation between increase in follicle size and increase in estradiol levels exists (Henderson et al., 1982), but during altrenogest treatment follicles increased in size up to Day 5 while estradiol levels decreased from Day 2 onwards. A similar uncoupling between follicle growth and estradiol production was described by Langendijk et al. (2009). They found that sows, that failed to respond with ovulation to an intermittent suckling regime, also showed an increase in follicle size without a rise in estradiol production. They hypothesized that this could be due to reduced follicular responsiveness to LH in these sows. During altrenogest treatment, estradiol production may either decrease because LH stimulation of the follicles decreases as treatment progresses (as the suppressive effect of altrenogest becomes stronger) or may decrease because LH responsiveness of the follicles becomes reduced, which could be related to insufficient LH receptor formation during follicle development.

In Chapter 7 follicle size was 4.4 mm at Day 5, declined about 0.7 mm in the following 1-2 days and remained relatively stable till end of treatment (average size 3.9 mm). Average estradiol levels declined for Day 2 till Day 5 and remained on this lower level for the rest of altrenogest treatment. However, the daily variation in estradiol release remained present.

A large individual variation in follicle development was seen between sows. Figure 1 shows individual follicle growth curves of 3 representative sows (Chapter 7). Some sows show a strong increase in follicle size followed by a decrease in follicle size during treatment (panel a and c), while others show a more or less stable follicle size throughout treatment (panel b).

The differences in follicular growth patterns may, as mentioned before, be caused by variation in LH release between animals and thus variation in the suppressive effect of altrenogest or a variation in the follicular responsiveness to LH between animals (Langendijk et al., 2009). When follicular responsiveness to LH differs, this may affect follicular development during, but also after altrenogest treatment. During altrenogest treatment gonadotropin levels are relatively low. Follicles that do not receive sufficient gonadotropin support will eventually go in atresia (Driancourt et al., 1987), so it seems reasonable to assume that follicles will become atretic at some point during treatment. When they become atretic may differ between follicle size classes as Driancourt et al., (1987) found that follicles that were large (> 2 mm) at the moment of hypophysectomy in hypophysectomized ewes stopped growing, but were able to survive for a longer period

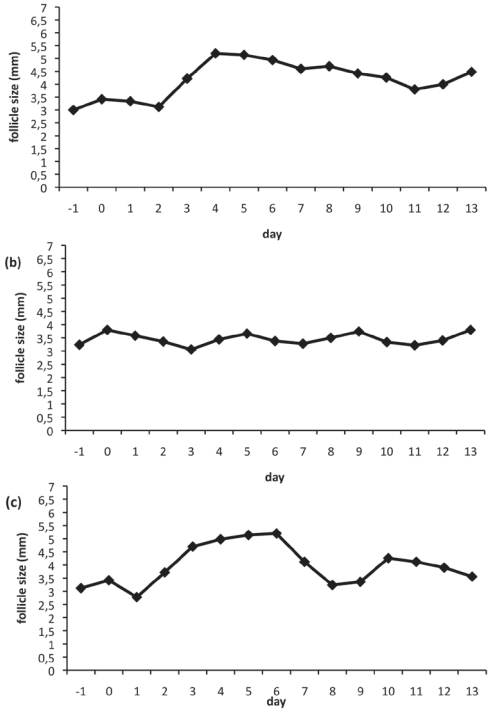

Figure 1. Different follicle growth patterns as seen during a 15 days altrenogest treatment for 3 representative sows. Weaning = Day 0.

of time without gonadotropic support than smaller follicles. Sows of Chapter 7 were classified as having high (≥ 0.58 ng/mL) or low (<0.58 ng/mL) levels of LH at weaning to investigate differences in subsequent follicular development. Surprisingly, no difference in follicle development was found between sows with high or low levels of LH at weaning (3.4 *vs.* 2.9 mm at weaning and 3.6 mm *vs.* 4.0 mm on Day 7, respectively.). This indicates that follicles of similar sizes may vary in their LH responsiveness.

In Figure 2 variation in LH responsiveness during altrenogest treatment is demonstrated. Sows were classified as low (a), intermediate (b) and highly (c) responsive to LH based on the daily variation in estradiol level during the second week of treatment. Estrogenic activity decreased almost completely (a), partially (b) or hardly (c) as treatment progressed, which seems to reflect a low (a), intermediate (b) or high (c) responsiveness to LH. This may indicate that (partial) atresia occurred in (a) and (b), because as follicles become atretic their responsiveness to both FSH and LH reduces (resulting in a decrease in estrogenic activity) as a result of a reduction in gonadotropin receptors (Carson et al., 1979). FSH levels and follicle sizes during altrenogest did not differ significantly for sows with low, intermediate and high responsiveness to LH (Figure 2). This shows that follicle size is not a valid indicator of follicle quality during altrenogest treatment. The variation in estrogenic activity seems to be related to the lactational burden, since sows with high estrogenic activity had lost less bodyweight and less back fat during lactation (Table 2). Also

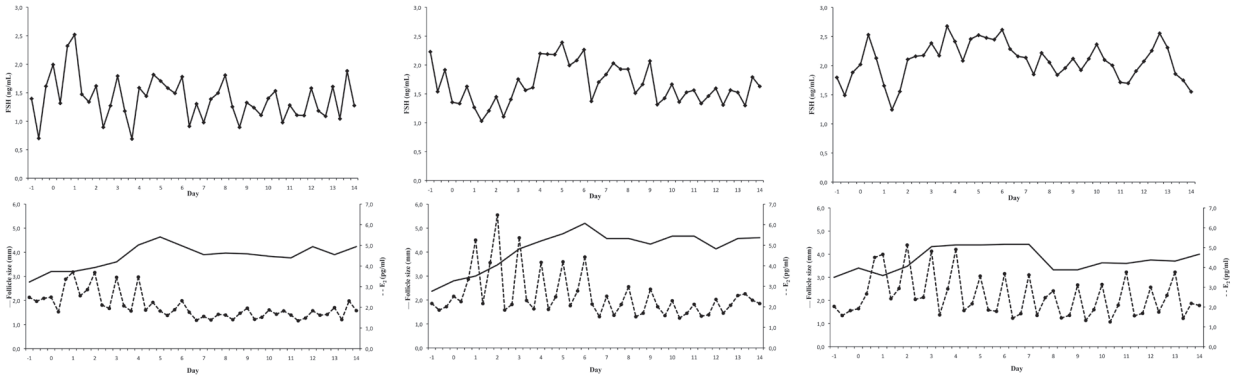

Figure 2. FSH levels (ng/mL) in top panels and follicle growth (mm) (solid line) and E_2 levels (pg/mL) (dotted line) in bottom panels for sows with low (a, n = 3), medium (b, n = 3) or high (c, n = 4) estrogenic activity during the second week (d7-d13) of altrenogest treatment.

LH levels tended to be higher at the day before weaning and at weaning for sows with high estrogenic activity (Table 2), probably as a result of a less severe lactational burden (Zak et al., 1997[b]). Normally the number of LH receptors present on the follicle, increases as follicle size increases (Liu et al., 2000), but a negative energy balance during lactation may reduce plasma insulin levels, which are necessary for LH receptor development (Poretsky and Kalin, 1987). So, low insulin levels may result in insufficient LH receptor development at weaning and thus a reduced responsiveness to LH. So, in sows with a less severe lactational burden this could have led to follicles that were better equipped (more LH receptors) to respond to the higher LH levels. Sows with high estrogenic activity during the second week of estrus also tended to have shorter intervals to estrus.

Table 2. Lactational parameters and subsequent reproductive performance for altrenogest treated sows with low, intermediate or high estrogenic activity during the second week (d7-d13) of altrenogest treatment.[1]

	Estrogenic activity			
Item	Low (n = 3)	Intermediate (n=3)	High (n = 4)	*P*-value
Average LH levels on D-1 (ng/mL)	0.35 ± 0.01	0.42 ± 0.05	0.51 ± 0.08	0.06
Average LH levels on D0 (ng/mL)	0.51 ± 0.07	0.56 ± 0.22	0.78 ± 0.13	0.08
Weight loss during lactation (kg)	25 ± 2	22 ± 7	9 ± 7	0.04
Back fat loss during lactation (mm)	7 ± 1	7 ± 2	2 ± 2	0.03
Onset to estrus (days)	6 ± 0.4	4 ± 0.5	4 ± 0.7	0.09

[1] Based on the level of variability in estradiol between 0700h and 1500h sampling at d7-d13

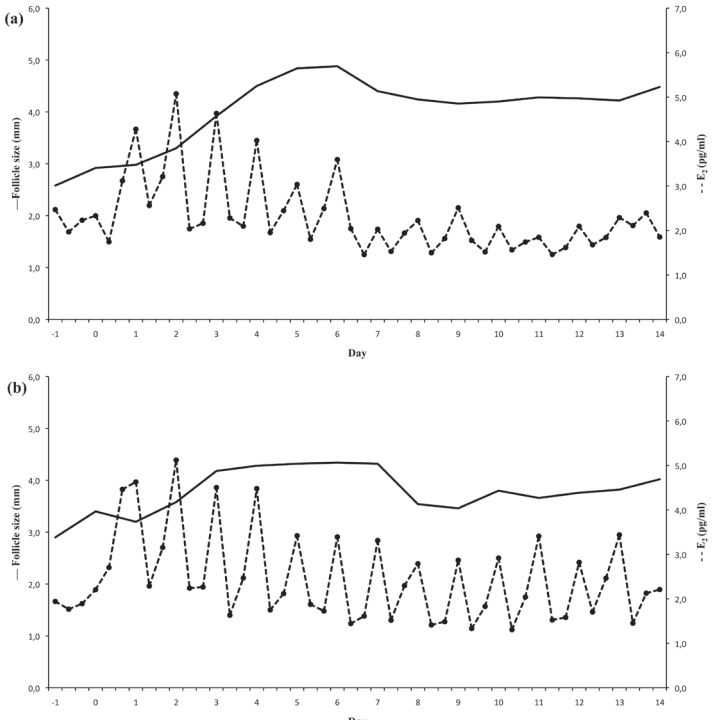

Figure 3. Follicle growth (mm) (solid line) and E_2 levels (pg/mL) (dotted line) during altrenogest treatment for sows with high (> 8%) weight loss (a) or low (≤ 8%) weight loss (b) during lactation.

Figure 3 shows follicle development and estrogenic activity for sows that had high (a) weight loss (> 8%) or low (b) weight loss (≤ 8%) during lactation. Sows that suffered a higher lactational burden had follicles that declined in size sooner (Day 6 *vs.* Day 7) and showed reduced estrogenic activity after this decline in follicle size, compared to sows with a low lactational burden. This indicates that in the latter group the old cohort of follicles may have been replaced by a new cohort of healthy (estrogenic) follicles while in the sows that suffered a higher lactational burden no healthy (estrogenic) cohort was recruited after the decline in follicle size, because very little estrogenic activity is seen till the end of treatment.

Average follicle size of sows with low, intermediate and high estrogenic activity in the second week of treatment reduced after Day 5, Day 6 and Day 7, respectively (Figure 2). This reduction in average follicle size, which coincided with a reduction in estrogenic activity, seems to indicate that follicles became atretic. The fact that in the sows with high estrogenic activity follicle size did not decline until Day 7 may indicate that follicles that are more responsive to LH indeed are better equipped to survive with low gonadotropin support before they become atretic.

In sows with high estrogenic activity, the reduction in follicle size was accompanied by only a small decrease in estrogenic activity, which suggests that the initial follicle pool was replaced by a new, LH responsive (healthy), follicle pool. This may also explain why there was interval to estrus was shorter in these sows and why high embryo survival rate was related to

high estrogenic activity on D13 (r = 0.7; *P* = 0.04). Interestingly, estrogenic activity on D13 was not related to lactational parameters or LH pulsatility around weaning.

From the above it becomes clear that there is a large variation between sows in response to altrenogest treatment (LH levels, follicle size, estrogenic activity) and that this may be related to lactational burden, which ultimately may affect the reproductive outcome of altrenogest treatments. As sows with similar follicle sizes, at weaning and during treatment, showed different levels of estrogenic activity, follicle size during altrenogest treatment is not a good indicator of follicle quality.

8.3 Altrenogest application strategy and subsequent fertility

Reproductive performance of sows can be positively affected, negatively affected or not affected by post weaning altrenogest treatments (for an overview see Table 3). Such treatments may vary in the moment at which treatment is started, in the duration of treatment and in the applied dose. The following paragraph discusses how application strategies may influence subsequent fertility.

8.3.1 Start and duration of altrenogest treatments

After weaning normally a rapid increase in LH pulsatility is seen (Shaw and Foxcroft, 1985), resulting in a short interval to estrus. Primiparous sows, especially ones with a more severe negative energy balance during lactation, may have low LH pulsatility around weaning, which is associated with a longer interval to estrus (van den Brand et al., 2000). Along with the selection for prolifacy also a direct or an indirect selection for short weaning to estrus intervals occurred (Quesnel, 2009). So, the modern sow is more likely to have a rapid increase in LH levels after weaning. Several studies showed that FSH levels also increase after weaning, but this effect was not seen in other studies (for an overview see Prunier et al., 2003). But Langendijk et al., (2009) found that on the day of weaning 50% of the LH pulses coincided with a FSH pulse, indicating that FSH levels, like LH, respond to the increase in GnRH release after weaning. Altrenogest provides a negative feedback on GnRH release from the hypothalamus, resulting in reduced LH and FSH release from the pituitary. Plasma peak levels of altrenogest are not reached before 3 to 6 hours after administration (Committee for Veterinary Medical Products, emea.europa). So, in order to minimize LH pulsatility after weaning it seems that altrenogest should be applied between 3-6 hours before weaning. Altrenogest is a progestagen and it is unclear whether accumulation of plasma altrenogest levels occurs. However, in Chapter 4 pre-weaning altrenogest treatment from 3 days before weaning onwards did not result in suppression of follicle growth before and after weaning, so if accumulation of altrenogest occurs then it takes longer than 3 days to reach more effective doses. If this is the case then treatment should be started earlier than 3 days before weaning to improve the effective dose around weaning. However, if accumulation of altrenogest occurs then also effects of duration

of treatment on follicle development after treatment and/or interval to estrus would be expected. There are no indications that those parameters are effected by duration of treatment, so it is more likely that no accumulation of altrenogest occurs, so initiating altrenogest treatment 3-6 hours before weaning would be sufficiently effective.

If altrenogest treatment is first applied at the moment of weaning than chances are that an initial increase in LH pulsatility (Shaw and Foxcroft, 1985; Quesnel and Prunier, 1995) is not prevented, resulting in the selection of (antral) follicles (Driancourt et al., 1995). The increase in LH pulsatility together with the increase in FSH levels will stimulate follicle growth. When treatment is initiated a day after weaning, then the suppressive effect of altrenogest on GnRH release is not established until at least 24 hours after weaning, which means that LH pulsatility increases after weaning, as normally occurs in untreated sows (Shaw and Foxcroft, 1985; Chapter 7). This results in recruitment of the follicle pool present at weaning and outgrowth of follicles before suppression of GnRH release is reached. Follicles that are more responsive to LH, or follicles that were more stimulated by LH around weaning, will develop more during altrenogest treatment and, as Figure 2 showed, will survive longer during a period of low gonadotropin support as the suppressive effect of altrenogest becomes stronger. It is not clear what it means for follicle quality to survive during low gonadotropin conditions, but as sows with low, intermediate and high responsiveness to LH (estrogenic activity) showed a decline in follicle size and reduced estrogenic activity after 5, 6 or 7 days, respectively (Figure 2) it indicates that follicle quality is affected by a few days with little gonadotropic support. This may subsequently affect their response to increased levels of LH after treatment withdrawal.

As follicles become atretic during altrenogest treatment they may be replaced by a new cohort of follicles that developed predominantly during a period of improved nutritional status after weaning (restored levels of insulin; van den Brand et al. 2000 and IGF-1; van den Brand et al., 2001), because it takes antral follicles around 2 weeks to grow from 0.4 mm to 3 mm (Morbeck et al., 1992). An improved nutritional status during follicle development may improve follicle and oocyte quality (Zak et al., 1997ab; Cosgrove 1998 Ferguson et al., 2003; Quesnel et al., 2009). That follicle quality indeed may be improved by 15 day altrenogest treatments follows from the higher progesterone levels (on day 10 of gestation), that were found compared to untreated controls (31 ± 1 *vs.* 22 ± 1 ng/ml, respectively; $P < 0.0001$; Chapter 7; *results not shown*). So, if treatment withdrawal occurs when a new cohort of follicles has developed, it may improve subsequent fertility. This is probably the reason why long altrenogest treatments of 12-15 days (Table 3) usually result in improved reproductive performance (ovulation rate: Koutsotheodoros et al., 1998, increased litter size: Koutsotheodoros et al., 1998, Patterson et al., 2008 and Chapter 3). Duration of treatment between 12 and 15 days is probably long enough to assure complete atresia of the antral follicle pool present at weaning and also long enough to overcome the negative effects of lactation. This indicates that in order to improve reproductive performance after altrenogest treatment, follicular atresia during altrenogest treatment may be necessary. A decrease in follicle size between Days 5 and 6, with altrenogest withdrawal at Day 6, was associated with higher embryo survival rates (Chapter 5). This decrease in size

Table 3. Reproductive performance after post weaning altrenogest treatments (Alt) compared to untreated controls (C).

Treatment					Results			
Start treatment	Lactation length	Parity	Dose (mg)	Length (days)	Weaning-Estrus Interval[a]		% Estrus[b]	
					C	Alt	C	
before weaning								
-48h	18	2-7	15	7	5.1	+1.7	78.8	
-48h	18	2-7	15	14	5.1	+2.2	78.8	
-36h	21	1	15	8	6.5	-1.9	-	
-36h	21	1	15	15	6.5	-1.3	-	
-36h	21	1	20	8	6.5	-1.8	-	
-36h	20	1	20	4	4.9	-1.4	89	
-36h	20	1	20	8	4.9	-0.6	89	
-36h	20	1	20	15	4.9	-1.1	89	
-36h	21	1	20	8	4.8	ns	87	
-36h	21	2-3	20	8	4.3	-0.7	100	
-36h	26	1	20	15	4.4	+0.5	-	
day of weaning								
0h	35	1	20	3	10.2	ns	92	
0h	35	1	20	7	10.2	ns	92	
0h	28	1	20	7	5.6	+9	94	
0h	35	1	30	7	10.0	ns	70	
+3h	21	1	20	5	4.4	-0.7	96.1	
+3h	21	1	20	5	4.4	-0.7	89.5	
after weaning								
+24h	12	1	20	12	7.3	-0.9	64	
+24h	22	1	20	5	6.9	ns	66	

1 Patterson et al., 2008, **2** Chapter 2, **3** Chapter 3, **4** Chapter 4, **5** Chapter 7, **6** Boland,1983, **7** Stevenson et al., 1985, **8** Kirkwood et al., 1986, **9** Werlang et al., *in press*; 2 different breeds were used in this study, **10** Koutsotheodoros et al., 1998; sows were weaned after 12 days of lactation, **11** Fernandez et al., 2005, [a] interval from weaning to estrus (controls) and from 1d after last altrenogest administration

		Ovulation Rate		Farrowing Rate		Litter Size		Ref
	Alt	C	Alt	C	Alt	C	Alt	
	+10	-	*	-	-	11.8	ns[c]	1
	-	-	-		-	11.8	+1.8[c]	1
	-	19.5	ns	-	-	-	-	2
	-	19.5	ns	-	-	-	-	2
	-	19.5	ns	-	-	-	-	2
	ns	-	-	89	ns	11.9	ns	3
	ns	-	-	89	ns	11.9	ns	3
	ns	-	-	89	ns	11.9	+2.5	3
	+13	-	-	88	ns	11.9	+1.5	4
	ns	-	-	93	ns	13.7	ns	4
	-	23.2	ns	-	-	16.7	ns[d]	5
	ns	-	-	-	-	10.5	ns	6
	ns	-	-	-	-	10.5	ns	6
	-30	-	-	46	+22	8.9	ns	7
	+30	-	-	-	-	10.7	ns	8
	-10	-	-	84	-14	11.1	-1.7	9
	ns	-	-	97	-30	10.7	ns	9
	+33	15.4	+1.5	-	-	10.3	+2.6[e]	10
	+20	-	-	-	-	12.3	ns	11

to estrus (altrenogest treated), [b] % of sows showing estrus after weaning (controls) or after altrenogest withdrawal (altrenogest treated), [c] Live fetuses on day 50 of gestation, [d] total number of embryos on day 10 of pregnancy, [e] viable embryos on day 25-28 of gestation, * significant increase of 3.4 ovulations was seen in parity 2-3 altrenogest sows compared to parity 2-3 controls

indicates that follicular atresia may have occurred, after which the old cohort was replaced with a new cohort of follicles resulting in improved embryo survival rates. Other sows did not show a decrease in follicle size between Days 5 and 6. In these sows, the old cohort of follicles, still being LH responsive, may have grown out to pre-ovulatory sizes and eventually ovulated after treatment withdrawal. This extended lifespan of relatively large follicles (4-5 mm) may reduce subsequent fertility (in cows: Townson et al., 2002), due to reduced oocyte competence (in cows: Revah and Butler, 1996). Therefore, it may not be desirable to ovulate follicles that remain present during altrenogest treatment unless treatment length is very short and follicle lifespan is not extended significantly.

Short altrenogest treatments (3-4 days) may not result in recruitment of new follicles, because withdrawal of treatment occurs before a decline in follicle size (atresia) is normally seen during altrenogest treatment (Day 5-7). So, little effect on subsequent reproductive performance is to be expected. However, it is possible that the improved nutritional status immediately after weaning positively affects follicle quality (Zak et al., 1997[ab], Cosgrove 1998) and thereby may lead to improved fertility after treatment withdrawal. Nevertheless, the short period of improved nutritional status may limit the effects. Indeed, no improved reproductive performance has been found after short altrenogest treatments (Table 3), but no adverse effects on reproductive performance have been reported either (Boland 1983; Chapter 3). Thus, the benefit of such short treatments may only be to synchronize estrus on a herd level for management purposes.

Contradicting results have been found for reproductive performance after altrenogest treatments of 5-8 days (Table 3). Some studies found improved reproductive performance (percentage of sows in estrus: Kirkwood 1986; Fernandez et al., 2005; Patterson et al., 2008; Chapter 4, farrowing rate: Stevenson et al., 1985 and litter size: Chapter 4) while other studies reported a negative effect on reproductive performance (percentage of sows in estrus: Stevenson et al., 1985; Werlang et al., *in press*, farrowing rate: Werlang et al., *in press* and litter size: Werlang et al., *in press*). Intermediate durations of treatment (5-8 days) may result in withdrawal of altrenogest treatment before follicles are completely atretic. Partially atretic follicles may still respond to the increased LH levels after altrenogest withdrawal and finally ovulate. As lifespan is extended in these follicles it seems reasonable to assume that follicle quality and oocyte competence can be reduced. Especially sows with follicles with high responsiveness to LH at initiation of treatment may be at risk for reduced fertility after 5-8 days treatment. Their follicles are likely able to survive for a longer period with low gonadotropin levels (during altrenogest treatment) and may therefore still be responsive to LH after treatment withdrawal. Combined data of Chapter 3 and 4 indeed show that sows with a high increase (>1.5mm) in follicle size (in other words: follicles, that responded well to the increase in FSH and LH levels after weaning) had lower pregnancy rates after 8 days treatment than sows with a low increase (≤ 1.5 mm) in follicle size (69%; 31 out of 45 *vs.* 85%: 46 out of 54, respectively; $P = 0.05$). Thus, for intermediate treatments to be effective atresia must have occurred before withdrawal of altrenogest. Atresia is affected by follicular development at weaning and follicle growth during

the first days of treatment. Positive effects on subsequent fertility are only expected in sows with follicles that show minimal LH responsiveness at weaning, which ensures that follicles go into atresia quickly when receiving little gonadotropin support, or in sows with minimal LH release at weaning. To achieve the latter, altrenogest should be applied 3-6 hours before weaning.

Studies that found a negative effect on reproductive performance (percentage of sows showing estrus: Stevenson et al., 1985; Werlang et al., *in press*, farrowing rate: Werlang et al., *in press* and litter size: Werlang et al., *in press*) started on the day of weaning (not specified at what time; Stevenson et al., 1985) and 3 hours after weaning (Werlang et al., *in press*). This relatively late start of altrenogest treatment may have caused an increase in LH pulsatility before the suppressive effect of altrenogest was established. This may have stimulated follicle growth immediately after weaning and as a result follicles developed further and survived for a longer period of time during altrenogest treatment. Depending on length of treatment, withdrawal may occur before atresia is complete, which can result in outgrowth and ovulation of these follicles, with negative effects for subsequent fertility. Koutsotheodoros et al. (1998) started altrenogest treatment 2 days after weaning and, surprisingly, found positive effects of treatment on subsequent fertility. Starting altrenogest treatment 2 days after weaning is expected to have a negative effect on subsequent reproductive performance, because the suppressive effect of altrenogest on GnRH release is not established until at least 48 h after weaning and the initial rise in LH and FSH levels could result in rapid outgrowth of the follicles after weaning. However, Koutsotheodoros et al. (1998) weaned sows already at day 14 of lactation. At this time, LH levels were likely to be very limited (Willis and Zak, 2003), which would explain why starting altrenogest treatment after weaning did not negatively affect reproductive performance in this study.

8.3.2 Dose of treatment

Altrenogest is registered to use at a daily dose of either 15 or 20 mg, depending on the country of registration. No dose depended studies have been performed to establish minimum required dose in sows, but both 15 mg and 20 mg have been found to synchronize estrus effectively. For gilts the minimum dose was found to be 10 mg (Kraeling et al., 1981). Lower doses may not suppress LH and FSH release sufficiently and stimulate follicle growth during altrenogest treatment as lower doses have been associated with an increase in the occurrence of cystic follicles (2.5 mg Redmer and Day, 1981). Also doses of 16 mg were seen to increase the incidence of cystic follicles (Kauffold et al., 2007), although, other studies did not find any relation between a dose of 15 mg in sows and an increased occurrence of cystic follicles in comparison to untreated controls (Patterson et al., 2008; van Leeuwen et al., 2010). No differences were found in follicle development, ovulation rate and subsequent early embryonic development between 15 and 20 mg (Chapter 2). Therefore, 15 mg and 20 mg are probably both adequate doses to use in sows if the administration method is precise enough. Altrenogest administration directly into the sow's mouth (dosing gun) is preferred over mixing the dose through the sows feed as the latter makes it more difficult to assure full ingestion of the dose. Nevertheless, no studies using 20 mg reported a higher inci-

dence of cystic follicles and this is the dose that is most commonly used in studies investigating the effects of altrenogest on subsequent fertility.

8.4 Sow related parameters and fertility after altrenogest treatments

Altrenogest treatments show a large variation between sows in their response; endocrine profiles, follicle development and reproductive performance. The question is can we predict the reproductive outcome of altrenogest treatment for specific sows? In Chapter 3 we found that follicle size at weaning affected the reproductive outcome of altrenogest treatments. In order to measure follicle size at weaning all sows could be routinely subjected to ultrasound at weaning, but in commercial set-up this is a very time consuming method. Therefore, the question rises; can we predict follicle size at weaning? Data of Chapter 3 and 4 were pooled to study relations between lactational parameters and follicle size at weaning. These data consisted of large numbers of sows, from the same farm and the same genetics. Figure 4 shows scatter plots that represent the relations between weaning weight (a), back fat depth at weaning (b), lactational weight loss in kg (c) and lactational weight loss as % of body weight (d) in relation to follicle size at weaning. Smaller follicles at weaning are expected for sows with a more negative energy balance during lactation (Kauffold et al., 2008; Quesnel et al., 2000). Also follicle quality at weaning of sows with a negative energy balance during lactation may be impaired (Zak et al., 1997b). Recruitment of these impaired follicles may have negative consequences for subsequent fertility: reduced ovulation rates (Hazeleger et al., 2005; Zak et al., 1997a), impaired embryo development (Algriany et al., 2004; Zak et al., 1997a) and increased embryo mortality (Almeida et al., 2000; Zak et al., 1997a).

Surprisingly, weight loss during lactation and sow weight and back fat depth at weaning were not related to follicle size at weaning (Figure 4). This may be explained by the relatively light lactational burden of these sows. These primiparous sows lost on average only 8% of their body weight during a 21 day lactation. Thaker and Bilkei (2005) found negative effects on reproductive performance (weaning to estrus interval, farrowing rate, litter size) in primiparous sows when weight loss exceeded 10% (Thaker and Bilkei, 2005). The relative large follicle size at weaning (3.7-3.9 mm) also indicates that the effects of lactation on follicle development were limited. The limited weight loss in our experiments may also explain why hardly any relations between lactational parameters and reproductive performance after altrenogest treatment were found. In primiparous sows, however, a positive relation between back fat at weaning and subsequent litter size was seen in untreated control sows, while this relation was absent in altrenogest sows. This shows that altrenogest improved reproductive performance in primiparous sows and especially in those with low body condition scores at weaning.

Application of post weaning altrenogest treatment in sows with limited weight loss during lactation or high body condition scores at weaning may actually result in a reduced subsequent fertility. Follicles of sows with limited weight loss during lactation may be highly responsive to LH at weaning because their follicles acquired sufficient LH receptors during development.

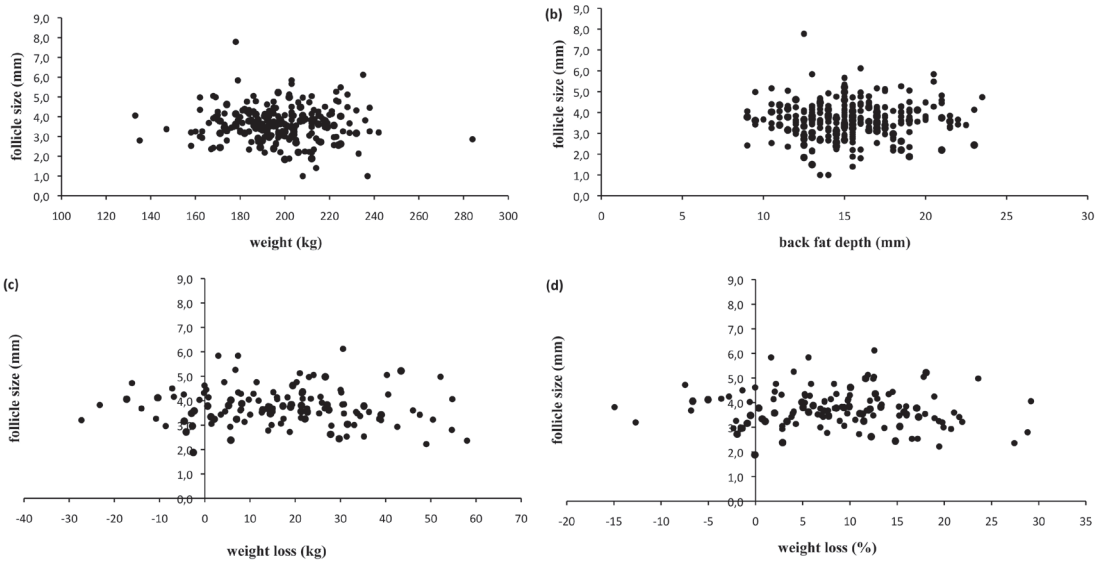

Figure 4. Weaning weight (a), back fat depth at weaning (b), weight loss during lactation in kg (c) and weight loss during lactation as % of body weight (d) in relation to average follicle size (five largest follicles) at weaning for primiparous sows.

The sows that showed high estrogenic activity during the second week of treatment (Figure 2) were sows that had lost less weight during lactation than sows that showed intermediate or low estrogenic activity. As can be seen from Figure 2, sows with high estrogenic activity in the second week of treatment also showed higher estrogenic activity after weaning than sows with low estrogenic activity. Because of the limited weight loss in sows with high estrogenic activity their FSH and LH levels around weaning were probably higher than in sows with low estrogenic activity (that lost more body weight during lactation) as a negative energy balance during lactation may affect gonadotropin levels and/or pulsatility around weaning (Zak et al., 1997b, Quesnel et al., 1998; van den Brand et al, 2000). This results in better equipped follicles to survive during conditions with low gonadotropin support, which may cause an extension of follicle lifespan and may have negative consequences for fertility (reduced embryo survival and reduced farrowing rates) after altrenogest treatment of 5-8 days.

Multiparous sows suffer less from a negative energy balance during lactation than primiparous sows and also the effects of weight loss during lactation on subsequent fertility are less severe (Thaker and Bilkei, 2005). Therefore, little improvement would be expected after post weaning altrenogest treatment on subsequent reproductive performance in multiparous sows. Indeed, in Chapter 4 no effect of altrenogest treatment was found on pregnancy rate, farrowing rate or litter size in multiparous sows (parity 2-3) compared to untreated multiparous control sows. Surprisingly, Patterson et al. (2008) found more viable fetuses on D50 of gestation after 14 days altrenogest treatment, but not after 7 days altrenogest treatment in multiparous sows (parity

2-7), which was attributed to improved follicle and oocyte treatment in the longer treatment due to the longer recovery period after weaning. There is no information on the lactational burden of sows in the study of Patterson et al. (2008), but the positive effect on reproductive performance in multiparous sows may also be related to improved nutritional status of the sows during long altrenogest treatment and the occurrence of follicle turnover during treatment, which improves follicle quality. Since no improved fertility in multiparous sows was found after 8 days of treatment (Patterson et al., 2008; Chapter 4), it seems that post weaning altrenogest treatment in multiparous sows may have a positive effect only when the recovery period is long enough. This may be related to improved follicle quality due to follicle turnover in long altrenogest treatments.

Additionally, lactational factors such as lactation length, number of weaned piglets and feed intake during lactation that affect the lactational burden (and thus follicle development during lactation) may all influence reproductive outcome after post weaning altrenogest treatment as well.

8.5 Recommendations for practical use of once-daily altrenogest treatment after weaning

To use once-daily, post weaning altrenogest treatment as a successful management tool to overcome the negative effects of first lactation, and thus to improve fertility in the second parity, one should take the following in consideration (for an overview see Table 4). Treatments should be initiated before weaning and altrenogest levels should reach peak levels at the moment of weaning to suppress an increase in FSH and LH levels immediately after weaning. Therefore, ideally, altrenogest should be administered 3-6 hours before weaning. Both treatments of 15 or 20 mg can be used, but as no detrimental effects on subsequent reproductive performance have been reported using 20 mg (the use of 15 mg has been reported to increase the incidence of cystic follicles) the higher dose probably results in a more adequate suppression of GnRH release and is therefore a more recommended dose to use. Long altrenogest treatments (12-15 days) maybe applied without any risk for reduced reproductive performance and herd fertility of primiparous sows (and maybe even multiparous sows) maybe improved by this strategy. However, a treatment of around 2 weeks increases the number of days NIP (Not In Pig) per sow and also treatment costs are higher than with shorter treatment periods. Intermediate treatments (5-8) days may improve reproductive performance, but should be applied only in sows that suffered a severe lactational burden and are in low body condition at weaning to lower the chances of reduced fertility after treatment. Shorter treatments (3-4 days) have not been reported to have a negative effect on subsequent reproductive performance, but positive effects of short treatment on subsequent reproductive performance are probably limiting.

Table 4. Recommendations for once-daily application of post weaning altrenogest in primiparous and multiparous sows that had high or low body condition loss during lactation[1]

	Treatment		
Animal group	Short (3-4 days)	Intermediate (5-8 days)	Long (12-15 days)
Primiparous			
Low body condition loss	Yes[2]	No[3]	Yes[4]
High body condition loss	Yes[2]	Yes	Yes[4]
Multiparous			
Low body condition loss	No[5]	No[5]	Yes[4]
High body condition loss	No[5]	Maybe[6]	Yes[4]

[1] On the day of weaning treatment should be administered 3-6 hours before weaning

[2] Little improvement in reproductive performance is to be expected

[3] May have negative consequences for reproductive performance

[4] Preferred application with most chances on reproductive performance

[5] No negative effects on reproductive performance are expected but no improvement either

[6] Application may improve reproductive performance

8.6 Recommendations for future research

This thesis improved the understanding of follicular and endocrine changes during altrenogest treatment. Nevertheless some questions remain unanswered. It is clear that follicle growth is stimulated during the first days of altrenogest treatment due to incomplete suppression of LH and FSH release. Depending on follicle responsiveness to LH this may have different effects on subsequent fertility, probably because of reduced follicle quality. It is however, not clear what exactly happens in the follicle and if/how this affects oocyte quality and quality of the corpora lutea developing from the follicles after ovulation. It would, therefore, be interesting to investigate follicular fluid contents of e.g. the 10 largest follicles per ovary at D0 (weaning), D4 (just before follicles may start to become atretic), D8 (immediately after atresia) and D12 (after follicle turnover) of sows that receive altrenogest treatment from D-1 till slaughter (D0, D4, D8 or D12). Follicular fluid could be analyzed on the E_2:testosterone ratio, to investigate gradations of atresia (Whitley et al., 1998) and levels of IGF (Whitley et al., 1998) and VEGF (Mattioli et al., 2001) could be measured to study follicle health. To determine follicle receptiveness to LH, LH receptor mRNA (Liu et al., 2000) could be analyzed. Oocyte maturation capacities could be measured in vitro (Hunter and Wiesak , 1990; Zak et al., 1997[a]) on day 3 after altrenogest withdrawal of sows that received no altrenogest, altrenogest till D4, till D8 and till D12. This would provide proof of the theory of follicular atresia and follicle turnover during post weaning altrenogest treatments.

Follicular atresia played an important role in the success of altrenogest treatments with intermediate duration (5-8 days). Withdrawal of treatment should occur after atresia is complete and as follicles go into atresia when receiving little gonadotropin support it would be interesting to study LH and FSH release when time between 2 altrenogest administrations is reduced. This should lead to a more severe suppression of LH and FSH release which may trigger follicular atresia sooner after weaning.

A large variation in follicle development was seen between sows, which seemed related to variation in LH responsiveness of the follicles. For practical applications it would be interesting to investigate whether LH responsiveness of sows can be predicted by lactational parameters or follicle characteristics and whether this leads to follicle variation within sows, which may explain effects on subsequent fertility (litter size).

Such studies should be performed in sows that vary in their lactational burden, because the lactational burden has long-lasting consequences for follicle quality during altrenogest treatments. Experiments should have a group of sows with a more severe lactational burden (that lost substantial body condition) than used in the current studies, because in those sows effects of altrenogest treatment on subsequent reproductive performance are expected to be greater.

8.7 Conclusion

The aim of this thesis was to develop a better understanding of the use of altrenogest after weaning as a strategy to overcome the negative effects of first lactation on follicle development and subsequent reproductive performance.

Responses to post weaning altrenogest treatment (follicle development, endocrine profiles and reproductive performance) are highly variable between sows and this is affected by application strategy (start and duration of treatment, dose) and sow related factors (parity, lactational burden).

An increase in follicle size during altrenogest treatment was consistently seen in all experiments. This is likely caused by an incomplete suppression of GnRH release during altrenogest treatment, resulting in LH and FSH release during a part of the 24 hours between daily altrenogest administrations. The suppressive effect of altrenogest on GnRH release increases in the period after lactation. This is probably related to a lower metabolism of altrenogest. The gonadotropin levels during sustained altrenogest treatment prevent outgrowth of the follicles to pre-ovulatory sizes. Instead, follicles show reduced estrogenic activity and finally go into atresia.

A treatment length of 12-15 days is long enough to assure atresia of the entire follicle pool. The atretic follicle pool is replaced by a new cohort of follicles, which predominantly developed during more favourable metabolic conditions and therefore may improve follicle and oocyte quality, resulting in improved reproductive performance.

Short altrenogest treatments (3-4 days) are too short to ensure follicular atresia before altrenogest withdrawal. Improvement of subsequent fertility as a result of follicular turnover is

therefore not to be expected, even though the improved nutritional status after weaning may directly improve follicle quality and thus subsequent fertility.

Altrenogest treatments of intermediate durations (5-8 days) may result in withdrawal of altrenogest treatment before follicles are completely atretic. The extended lifespan of these follicles may negatively affect follicle quality and oocyte competence. These intermediate treatments may improve subsequent fertility when follicle growth during treatment is limited by adequate suppression of GnRH release and should only be applied in sows with follicles that show little responsiveness to LH and/or in sows that have low LH levels at weaning.

References

Algriany O., Bevers M., Schoevers E., Colenbrander B., Dieleman S., 2004. Follicle size-dependent effects of sow follicular fluid on in vitro cumulus expansion, nuclear maturation and blastocyst formation of sow cumulus oocytes complexes. Theriogenology 62(8): 1483-1497.

Almeida F.R.C.L., Kirkwood R.N., Aherne F.X., Foxcroft G.R., 2000. Consequences of different patterns of feed intake during oestrous cycle in gilts on subsequent fertility. J. Anim. Sci. 78: 1556-1563.

Belstra B.A., Flowers W.L., See M.T., 2004. Factors affecting temporal relationships between estrus and ovulation in commercial sow farms. Anim. Rep. Sci. 84:377-394.

Blondin P, Sirard MA, 1995: Oocyte and follicular morphology as determiningcharacteristics for developmental competence in bovine oocytes. Mol. Reprod. Dev. 41: 54-62.

Bo G.A., Adams G.P., Caccia M., Martinez M., Pierson R.A., Mapletof R.J., 1995. Ovarian follicular wave emergence after treatment with progestagen and estradiol in cattle. Anim. Reprod. Sci. 39: 193-204.

Boland M.P., 1983. Control of oestrus in primiparous sows. Theriogenology 19: 377-384.

Bracken C.J, Lamberson W.R., Safranski T.J., Lucy M.C., 2003. Factors affecting follicular populations on Day 3 postweaning and interval to ovulation in a commercial sow herd. Theriogenology 60: 11-20.

Brand van den H., Dieleman S.J., Soede N.M., Kemp B., 2000. Dietary energy source at two feeding levels during lactation of primiparous sows: I. Effects on glucose, insulin, and luteinizing hormone and on follicle development, weaning-to-estrus interval, and ovulation rate. J. Anim. Sci. 78: 396-404.

Brand van den H., Prunier A., Soede N.M., Kemp B., 2001. In primiparous sows, plasma insulin-like growth factor-I can be affected by lactational feed intake and dietary energy source and is associated with luteinizing hormone. Reprod. Nutr. Dev. 41: 27-39.

Britt J.H., Armstrong J.D., Cox N.M., Esbenshade K.L., 1985. Control of follicular development during and after lactation in sows. J. Reprod. Fert., Suppl. 33: 37-54.

Burger L.L., Dalkin A.C., Aylor K.W., Haisenleder D.J., Marshall J.C., 2002. GnRH pulse frequency modulation of gonadotropin subunit gene transcription in normal gonadotropes – Assessment by primary transcript assay provides evidence for roles of GnRH and follistatin. Endocrinology 143: 3243-3249.

Burke C.R., Mihm M., Macmillan K.L., Roche J.F., 1994. Some effects of prematurely elevated concentrations of progesterone on luteal and follicular characteristics during the oestrous cycle in heifers. Anim. Reprod. Sci. 35: 27-39.

Cardenas H. and Pope W.F., 2002. Control of ovulation in swine. J. Anim. Sci. 80: E36-E40.

Carson R.S., Findlay J.K., Burger H.G., Trounson A.O., 1979. Gonadotropin receptors of the ovine ovarian follicle during follicular growth and atresia. Biol. Reprod. 21: 75-87.

Christianson W.T., 1992. Stillbirths, mummies, abortions, and early embryonic death. Vet. Clin. North Am., Food Animal Practice (8) 3: 623-639.

Clowes E.J., Aherne F.X., Foxcroft G.R., 1994. Effect of delayed breeding on endocrinology and fecundity of sows. J. Anim. Sci. 72: 283-291.

Clowes E.J., Williams I.H., Baracos V.E., Pluske J.R., Cegielski A.C., Zak L.J.,
Aherne F.X., 1998. Feeding lactating primiparous sows to establish three divergent metabolic states: II. Effect of nitrogen partitioning and skeletal muscle composition. J. Anim. Sci. 76:1154–1164.

Clowes, E.J., Aherne, F.X., Schaefer, A.L., Foxcroft, G.R., Baracos, V.E., 2003[a]. Parturition body size and body protein loss during lactation influence performance during lactation and ovarian function at weaning in first-parity sows. J. Anim. Sci. 81: 1517–1528.

Clowes E.J., Aherne F.X., Foxcroft G.R., Baracos V.E., 2003[b]. Selective protein loss in lactating sows is associated with reduced litter growth and ovarian function. J. Anim. Sci. 81: 753–764.

Cosgrove J.R., Booth P.J., Foxcroft G.R., 1991. Opioidergic control of gonadotrophin secretion in the prepubertal gilt during restricted feeding and realimentation. J. Reprod. Fert. 91 : 277-284.

Cosgrove J.R. 1998. Nutrition / endocrine interactions. In: Wiseman J., Varley M.A.,
Chadwick J.P., Progress in Pig Science. Nottingham University Press, Nottingham, UK. pp35-36.

Dalkin A.C., Haisenleder D.J., Ortolano G.A., Ellis T.R., Marshall J.C., 1989. The frequency of gonadotropin-releasing hormone stimulation differentially regulates gonadotropin subunit messenger ribonucleic acid expression. Endocrinology 125: 917-924.

Davis D.L., Knight J.W., Killian D.B., Day B.N., 1979. Control of estrus in gilts with a progestogen. J. Anim. Sci. 49: 1506-1509.

De Rensis F., Hunter M.G., Foxcroft G.R., 1993. Suckling-induced inhibition of luteinizing hormone secretion and follicular development in the early postpartum sow. Biol. Reprod. 48: 964-969.

Ding J. and Foxcroft G.R., 1994. Conditioned media produced by follicular shells of different maturity affect maturation of pig oocytes. Biol. Reprod. 50: 1377-1384.

Driancourt M.A., Fry R.C., Clarke I.J., Cahill L.P., 1987. Follicular growth and regression during the 8 days after hypophysectomy in sheep. J. Reprod. Fert. 79: 635-641.

Driancourt M.A., Locatelli A., Prunier A., 1995. Effects of gonadotropin deprivation on follicular growth in gilts. Reprod. Nutr. Dev. 35: 663-673.

Dusza L., Okrasa S., Ciereszko R., Kotwicaand G., Tilton J.E., 1988. Endocrine changes associated with spontaneous luteolysis in sows. I. temporal relationships among prolactin, prostaglandin F2α, progesterone and LH. Anim. Reprod. Sci. 17, 115-122. EMEA (European Medicines Agency; Veterinary Medicines and Inspections), 2005. Altrenogest; summary report. EMEA/MRL/904/04-FINAL.

Everaert N., Vanderhaeghe C., Mateusen B., Dewulf J., van Soom A., de Kruif A., Maes D., 2007. Effects of post-weaning altrenogest treatment in primiparous sows. Effect van altrenogesttoediening na het spenen aan primipare zeugen. Vlaams Diergeneeskundig Tijdschrift 76: 293-299.

Everts H. and Dekker R.A., 1994. Balance trials and comparative slaughtering in breeding sows: description of techniques and observed accuracy. Livestock Prod. Sci. 37: 339-352.

Ferguson E.M., Ashworth C.J., Edwards S.A., Hawkins N., Hepburn N., Hunter M.G., 2003. Effect of different nutritional regimens before ovulation on plasma concentrations of metabolic and reproductive hormones and oocyte maturation in gilts. Reprod. 126:61-71.

Fernandez L., Diez C., Ordoñez J.M., Carbajo M., 2005. Reproductive performance in primiparous sows after postweaning treatment with a progestagen. J. Swine health Prod. 13: 28-30.

Foxcroft G. R. and Hunter M. G. (1985). Basic physiology of follicular maturation in the pig. J. Reprod. Fertil. Suppl. 33: 1–19.

Foxcroft G.R., Dixon W.T., Novak S., Putman C.T., Town S.C., Vinsky M.D.A., 2006. The biological basis for prenatal programming of postnatal performance in pigs. J. Anim. Sci. 84(E. Suppl.) Accessed Nov. 23, 2010. http://jas.fass.org.ezproxy.library.wur.nl/cgi/reprint/84/13_suppl/E105.

Flowers W.L., 2002. Control of reproduction in swine with a synthetic progestagen, Matrix®. Technical review paper available through Intervet, USA.

Gonzalez-Añover P., Encinas T., Gomez-Izquierdo E., Sanz E., Sanchez-Sanchez R.,

Gonzalez-Bulnes A., 2009. Accuracy of in vivo and ex vivo ultrasonographic evaluation of ovarian follicles and corpora lutea in sows. Theriogenology 71: 1433-1439.

Guthrie H.D., Ireland J.L.H., Good T.E.M., Ireland J.J., 1997. Expression of Different Molecular Mass Forms of Inhibin in Atretic and Nonatretic Follicles during the Early Luteal Phase and Altrenogest-Synchronized Follicular Phase in Pigs. Biol. Reprod. 56: 870-877.

Guthrie H.D. 2005. The follicular phase in pigs: Follicle populations, circulating hormones, follicle factors and oocytes. J. Anim. Sci. 83: E79-89.

Hazeleger W., Soede N.M., Kemp B., 2005. The effect of feeding strategy during the pre-follicular phase on subsequent follicular development in the pig. Dom. Anim. Endocrinol. 29: 362-370.

Henderson K.M., McNeilly A.S.,Swanston I.A., 1982. Gonadotrophin and steroid concentrations in bovine follicular fluid and their relationship to follicle size J. Reprod. Fert. 65: 467-473.

Hoving L.L., Soede N.M., Graat E.A.M., Feitsma H., Kemp B., 2010. Effect of live weight development and reproduction in first parity on reproductive performance of second parity sows. Anim. Reprod. Sci. 122:82-89.

Hoving L.L., Soede N.M., Graat E.A.M., Feitsma H., Kemp B., 2011. Reproductive performance of second parity sows: relations with subsequent reproduction. Livestock Sci. doi:10.1016/j.livsci.2011.02.019.

Hunter M.G., Wiesak T., 1990. Evidence for and implications of follicular heterogeneity in pigs. J. Reprod. Fertil. Suppl. 40: 163-177.

Itoh T., Kacchi M., Abe H., Sendai Y., Hoshi H., 2002. Growth, antrum formation and estradiol production of bovine preantral follicles cultured in a serum free medium. Biol. Reprod. 67: 1099-1105.

Jong de FH, 1988: Inhibin, Physiological reviews 68: 555-607.

Kauffold J., Beckjunker J., Kanora A., Zaremba W., 2007. Synchronization of estrus and ovulation in sows not conceiving in a scheduled fixed-time insemination program. Anim. Reprod. Sci. 97: 84-93.

Kauffold J., Gottschalk J., Schneider F., Beynon N., Wähner M., 2008. Effects of feeding level during lactation on FSH and LH secretion patterns and follicular development in primiparous sows. Reprod. Dom. Anim. 43: 234-238.

Kemp B., Soede N.M., Hazeleger W., 1998. Control of ovulation. In: Wiseman J., Varley M.A., Chadwick J.P., Progress in Pig Science. Nottingham University Press, Nottingham, UK Cap 13.

Kirkwood R.N., Smith W.C., Lapwood K.R., 1986. Influence of oral administration of allyltrenbolone on subsequent litter size of primiparous sows. NZ Experimental Agriculture14: 477-480.

Kluivers-Poodt M., Gerritsen R., van Nes A., Langendijk P., 2010. Cortisol profiles in sows submitted to an intermitten suckling regime compared with that of abruptly weaned sows. Reprod. Dom. Anim. 45: 377–382.

Knox R.V. and Zas S.L.R., 2001. Factors influencing estrus and ovulation in weaned sows as determined by transrectal ultrasound. J. Anim. Sci. 79: 2957-2963.

Koketsu Y., Takahashi H., Akachi K., 1999. Longevity, lifetime pig production and productivity and age at first conception in a cohort of gilts observed over six years on commercial farms. J. Vet. Med. Sci. 61: 1001-1005.

Koutsotheodoros F., Hughes P.E., Parr R.A., Dunshea F.R., Fry R.C., Tilton J.E., 1998. The effects of post-weaning progestagen treatment (Regumate) of early-weaned primiparous sows on subsequent reproductive performance. Anim. Reprod. Sci. 52: 71-79.

Kraeling R.R., Dziuk P.J., Pursel V.G., Rampacek G.B., Webel S.K., 1981. Synchronization of estrus in swine with allyl trenbolone (RU2267). J. Anim. Sci. 52: 831-835.

Kunavongkrit A., Einarsson S., Settergren I., 1982. Follicular development in primiparous lactating sows. Anim. Reprod. Sci. 5:47-56.

Langendijk P., Dieleman S.J., Gerritsen R., Hazeleger W., Mainsant M., Soede N.M., Kemp B., 2007[a]. Pulsatile release of luteinising hormone during the luteal phase in lactating and weaned sows. Reproduction, Fertility and Development 19, 961-966.

Langendijk P., Dieleman S.J., van den Ham C.M., Hazeleger W., Soede N.M., Kemp B., 2007[b]. LH pulsatile release patterns, follicular growth and function during repetitive periods of suckling and non-suckling in sows. Theriogenology 67: 1076-1086.

Langendijk P., van den Brand H., Gerritsen R., Quesnel H., Soede N.M., Kemp B., 2008. Porcine luteal function in relation to IGF-1 levels following ovulation during lactation o rafter weaning. Reprod. Dom. Anim. 43: 131–136.

Leenhouwers J.I., Knol E.F., de Groot P.N., Vos H., van der Lende T., 2002. Fetal development in the pig in relation to genetic merit for piglet survival. J. Anim. Sci. 80: 1759-1770.

Leeuwen van J.J.J., Williams S.I., Kemp B., Soede N.M., 2010. Post-weaning Altrenogest treatment in primiparous sows; the effect of duration and dosage on follicular development and consequences for early pregnancy. Anim. Repro. Sci.119: 258-264

Leeuwen van J.J.J., Williams S.I., Martens M.R.T.M., Jourquin J., Driancourt M.A., Kemp B., Soede N.M., 2011[a]. The effect of different post-weaning altrenogest treatments of primiparous sows on follicular development, pregnancy rates and litter sizes. J. Anim. Sci. 89: 397-403.

Leeuwen van J.J.J., Martens M.R.T.M., Jourquin J., Driancourt M.A., Kemp B., Soede N.M., 2011[b]. Variation in LH pulsatility during 24 hours after a postweaning altrenogest treatment in relation to follicle development in primiparous sows. Anim. Reprod. Sci. 126: 101–107.

Leeuwen van J.J.J., Martens M.R.T.M., Jourquin J., Driancourt M.A., Kemp B., Soede N.M., 2011[c]. Effects of altrenogest treatments before and after weaning on follicular development, farrowing rate, and litter size in sows J. Anim Sci. 89: 2397-2406.

Liu J., Koenigsfeld A.T., Cantley T.C., Boyd C.K., Kobayashi Y., Lucy M.C., 2000. Growth and initiation of steroidgenesis in porcine follicles are associated with unique patterns of gene expression for individual components of the ovarian insulin-like growth factor system. Biol. Reprod. 63: 942-952.

Lonergan P., Monaghan P., Rizos D., Boland M.P., Gordon I., 1994. Effect of follicle size on bovine oocyte quality and developmental competence following maturation, fertilization and culture in vitro. Mol. Reprod. Develop. 37: 48-53.

Lucy M.C, Liu J., Boyd C.K., Bracken C.J., 2001. Ovarian follicular growth in sows. Reproduction Suppl. 58: 31-45.

Madej A., Lang A., Brandt Y., Kindahl H., Madsen M.T., Einarsson S., 2005. Factors regulating ovarian function in pigs. Dom. Anim. Endocrin. 29: 347-361.

Martinat- Botté F., Bariteau F., Badouard B., Terqui T., 1985. Control of pig reproductionin a breeding programme. J. Reprod. Fert., Suppl. 33: 211-228.

Martinat- Botté F., Bariteau F., Forgerit Y., Macar C., Moreau A., Terqui M., Signoret J.P., 1990. Control of oestrus in gilts II. Synchronization of oestrus with a progestagen, altrenogest (Regumate): effect on fertility and litter size. Anim. Reprod. Sci. 22: 227-233.

Martinat-Botté F., Bariteau F., Forgerit Y., Macar C., Poirier P., Terqui M., 1994. Control of reproduction with a progestagen-Altrenogest (Regumate) in gilts and at weaning in primiparous sows: effect on fertility and litter size. Reprod. Dom. Anim. 29(5): 362-365.

Martinat-Botté F., 1995. Synchronization of oestrus in gilts with Altrenogest: effects on ovulation rate and foetal survival. Anim. Reprod. Sci. 39: 267-274.

Mattioli M., Barboni B.,Turriani M., Galeati G., Zannoni A. Castellani G., Berardinelli P. Scapolo P.A., 2001. Follicle Activation Involves Vascular Endothelial Growth Factor Production and Increased Blood Vessel Extension. Biol. Reprod. 65: 1014-1019.

Miller H.M., Foxcroft G.R., Squires J., Aherne F.X., 1999. The effects of feed intake and body fatness on progesterone metabolism in ovariectomized gilts. J. Anim. Sci. 77: 3253–3261

Morbeck D.E., Esbenshade K.L., Flowers W. L., Britt J.H., 1992. Kinetics of follicle growth in the prepubertal gilt.. Biol. Reprod. 47: 485-491.

Morrow W.E.M., Leman A.D., Williamson N.B., Moser R., Pijoan, C., 1989. Improving parity-two litter size in swine. J. Anim. Sci. 67: 1707-1713.

Morrow W.E.M., Leman A.D., Marsh W.E., Williamson N.B., 1990. An economic study of lifetime piglet production for sows allocated to treatments designed to improve parity 2 litter size. Prev. Vet. Med. 10: 105-118.

Murdoch W.J, Van Kirk E.A., 1998. Luteal dysfunction in ewes induced to ovulate early in the follicular phase. Endocrinology 139: 3840-3484.

Patterson J., Wellen A., Hahn M., Pasternak A., Lowe J., DeHaas S., Kraus D., Williams N., Foxcroft G.R., 2008. Responses to delayed estrus after weaning in sows using oral progestagen treatment. J. Anim. Sci. 86: 1996-2004.

Perry G.A., Smith M.F., Lucy M.C., Green J.A, Parks T.E., MacNeil M.D, Roberts A.J., Geary T.W., 2005. Relationship between follicle size at insemination and pregnancy success. Proceedings of the National Academy of Sciences of the USA 102(14): 5268-5273.

Poretsky L. and Kalin M.F., 1987. The gonadotropic function of insulin. Endocr. Rev. 8: 132–141.

Prunier A., Messias de Braganqa M., Le Dividich J., 1997. Influence of high ambient temperature on performance of reproductive sows. Livest. Prod. Sci. 52:123-133.

Prunier A., Soede N., Quesnel H., Kemp B., 2003. Productivity and longevity of weaned sows. In: Pluske J.R., Le Dividich J., Verstegen M.W.A., Weaning the pig; Concepts and consequences. Wageningen Pers, Wageningen, the Netherlands. Cap 15.

Quesnel H., and Prunier A., 1995. Endocrine bases of lactational anoestrus in the sow. Reprod. Nutr. Dev. 35: 395-414.

Quesnel H., Paquier A., Mounier A.M. Prunier A., 1998. Influence of feed restriction during lactation on gonadotropic hormones and ovarian development in primiparous sows. J. Anim. Sci. 76: 856-863.

Quesnel H., Pasquier A., Mounier A., Prunier A., 2000. Feed restriction in cyclic gilts: gonadotropin-independent effects on follicular growth. Reprod. Nutr. Develop. 40(4): 405-414.

Quesnel H., Etienne M., Père M.C., 2007. Inflence of littersize on metabolic status and reproductive axis in primiparous sows. J. Anim. Sci. 85: 118–128.

Quesnel H., 2009. Nutritional and lactational effects on follicular development in the pig. Society of reproduction and fertility supplement 66: 121-134.

Ramírez O., Tomàs A., Barragan C., Noguera J.L., Amills M., Varona L., 2001. Pig melatonin receptor 1a (MTNR1A) genotype is associated with seasonal variation of sow litter size. Anim. Reprod. Sci. 115: 317-322.

Redmer D., Day B., 1981.Ovarian Activity and Hormonal Patterns in Gilts Fed Allyl Trenbolone. J. Anim. Sci. 53: 1088-1094. van Rens B.T.T.M., van der Lende T., 2002. Piglet and placental traits at term in relation to the estrogen receptor genotype in gilts. Theriogenology 57: 1651-1667.van Rens B.T.T.M., de Koning G., Bergsma R., van der Lende T., 2005. Preweaning piglet mortality in relation to placental efficiency. J. Anim. Sci. 83: 144-151.

Revah I., and Butler W.R., 1996. Prolonged dominance of follicles and reduced viability of bovine oocytes. J. Reprod. Fertil. 106: 39–47.

Salamone D.F., Adams G.P., Mapletoft R.J., 1999. Changes in the cumulus-oocyte complex of subordinate follicles relative to follicular wave status in cattle. Theriogenology 52: 549-561.dos Santos J.M.G., Wentz I., Bortolozzo F.P., Barioni, W., 2004. Early-weaned sows: Altrenogest therapy, estrus, ovulation, and reproductive performance. Anim. Reprod. Sci. 84: 407–413.

Sasaki Y., Koketsu Y., 2008. Sows having high lifetime efficiency and high longevity associated with herd productivity in commercial herds. Livest. Sci. 118: 140–146.

Savio J.D., Thatcher W.W., Badinga L., De la Sota R.L., Wolfenson D., 1993. Regulation of dominant follicle turnover during the oestrous cycle in cows. J. Reprod. Fertil. 97: 197-203.

Schwarz, T., Kopyra M., Nowicki J., 2008. Physiological mechanisms of ovarian follicular growth in pig - a review. Acta Veterinaria Hungarica 56: 369-378.

Serafim M.K.B., Araújo V.R., Silva G.M., Duarte A.B.G., Almeida A.P., Chaves R.N., Campello C.C., Lopes C.A.P., de Figueiredo J.R., da Silva L.D.M., 2010. Canine preantral follicles cultured with various concentrations of follicle-stimulating hormone (FSH). Theriogenology 74: 749-755.

Shaw H.J. and Foxcroft G.R., 1985. Relationships between LH, FSH and prolactin and reproductive activity in the weaned sow. J. Reprod. Fertil. 7517-28.

Sirard M.A., Blondin P., 1996. Oocyte maturation and IVF in cattle. Proc 13th Int. Congr. Anim. Reprod.: Research and Practice 42: 417-426.

Sirois J. and Fortune J.E., 1990. Lengthening the bovine estrous cycle with low levels of exogenous progesterone: A model for studying ovarian follicular dominance. Endocrinology 127: 916-925.

Soede N.M., Wetzels C.C., Zondag W., de Koning M.A.I., Kemp B., 1995. Effects of time of insemination relative to ovulation, as determined by ultrasonography, on fertilization rate and accessory sperm count in sows. J. Reprod. Fertil.104: 99-106.

Soede N.M., Helmond F.A., Schouten W.G.P., Kemp B., 1997. Oestrus, ovulation and peri-ovulatory hormone profiles in tethered loose-housed sows. Anim. Reprod. Sci. 46: 133-148.

Soede N.M., Hazeleger W., Kemp B., 1998. Follicle size and the process of ovulation as studied with ultrasound. Reprod. Dom. Anim. 33: 239-244.

Soede N.M., Bouwman E.G., Langendijk P., van der Laan I., Kanora A., Kemp B., 2007. Follicle Development during Luteal Phase and Altrenogest Treatment in Pigs. Reprod. Dom. Anim. 42: 329–332.

Soede N.M., Hazeleger W., Gerritsen R., Langendijk P., Kemp B., 2009. Ovarian responses to lactation management strategies. J. Reprod. Fert. Suppl. 66: 177-186.

Stevenson J.S. and Davis D.L., 1982. Estrous synchronization and fertility in gilts after 14 or 18 day feeding of altrenogest beginning at estrus or diestrus. J. Anim. Sci. 55: 119-123.

Stevenson J.S., Davis D.L., Pollmann D.S., 1985. Altrenogest and fat for summer breeding of primiparous sows. J. Anim. Sci. 61: 480-486.

Stock A.E. and Fortune J.E., 1993. Ovarian follicular dominance in cattle: relationship between prolonged growth of the ovulatory follicle and endocrine parameters. Endocrinology 132: 1108-1114.

Tast A., Love R.J., Evans G., Telsfer S., Giles R., Nicholls P., Voultsios A., Kennaway D.J., 2001. The pattern of melatonin secretion is rhythmic in the domestic pig and responds rapidly to changes in daylength. J. Pineal Res. 31: 294–300.

Thaker M., Bilkei G., 2005. Lactation weight loss influences subsequent reproductive performance of sows. Anim. Reprod. Sci. 88: 309-318.

Townson D.H., Tsang P.C., Butler W.R. Frajblat M. Griel L.C. Johnson C.J. Milvae R.A. Niksic G.M., Pate J.L., 2002. Relationship of fertility to ovarian follicular waves before breeding in dairy cows. J. Anim. Sci. 80: 1053-1058.

Tummaruk P., Lundeheim N., Einarsson S., Dalin A.M., 2001. Reproductive performance of purebred Hampshire sows in Sweden. Livest. Prod. Sci. 68: 67-77.

Varley M.A.,1983. Regulation of estrous cycles in groups of post-pubertal female pigs using allyl-trenbolone. Anim. Prod. Sci. 36: 211-215.

Vesseur P.C., Kemp B., Den Hartog L.A., 1997. Reproductive performance of the primiparous sow, the key to improve the efficiency of farm production | [Az elohasi koca reprodukciós teljesítménye - A sertéstelepek hatékonyságának növelésében játszott dönto szerepe] Magyar Allatorvosok Lapja 119: 629-634.

Virolainen J.V., Love R.J., Tasta A., Peltoniemi O.A.T., 2005. Plasma progesterone concentration depends on sampling site in pigs. Anim. Reprod. Sci. 86: 305-316.

Wagner A., Messe N., Bergmann M., Lekhkota O., Claus R., 2006. Effects of estradiol infusion in GnRH Immunized Boars on spermatogenesis. J. Androl. 27: 880-889.

Webb R., Garnsworthy P.C., Campbell B.K., Hunter M.G., 2007. Intra-ovarian regulation of follicular development and oocyte competence in farm animals. Theriogenology 68S: S22-S29.

Werlang R.F., Argenti1 L.E., Fries H.C.C., Bernardi M.L., Wentz I., Bortolozzo F.P., *in press*. Effects of Breeding at the Second Oestrus or After Post-Weaning Hormonal Treatment with Altrenogest on Subsequent Reproductive Performance of Primiparous Sows. Reprod Dom Anim doi: 10.1111/j.1439-0531.2010.01747.

Whitley N.C., Moore A.B., Cox N.M., 1998. Comparative effects of insulin and porcine somatotropin on postweaning follicular development in primiparous sows. J. Anim. Sci. 76: 1455-1462.

Willis H.J., Zak L.J., 2003. Duration of lactation, endocrine and metabolic state, and fertility of primiparous sows. J. Anim. Sci. 81: 2088-2102.

Yang H., Pettigrew J. E., Johnston L. J., Shurson G.C., Wheaton J. E., White Koketsu Y., Sower A.F., Rathmachers J.A., 2000. Effects of dietary lysine intake during lactation on blood metabolites, hormones, and reproductive performance in primiparous sows. J. Anim. Sci. 78: 1001-1009.

Zak L.J., Xu X., Hardin R.T., Foxcroft G.R., 1997[a]. Impact of different patterns of feed intake during lactation in the primiparous sow on follicular development and oocyte maturation. J. Reprod. Fertil. 110: 99-106.

Zak L.J, Cosgrove J.R, Aherne F.X., Foxcroft G.R.., 1997[b]. Pattern of feed intake and associated metabolic and endocrine changes differentially affect post-weaning fertility in primiparous lactating sows. J. Anim. Sci. 75: 208-216.

Zak L.J., Foxcroft G.R., Aherne F.X., Kirkwood R.N., 2008: Role of luteinizing hormone in primiparous sow responses to split weaning. Reprod. Dom. Anim. 43: 445-450.

Summary

Primiparous sows may suffer from suboptimal reproductive performance in the second litter, which is expressed as an increased weaning-to-estrus interval, reduced litter size and low farrowing rates. This reduced fertility in the second litter is also known as "the second litter syndrome". This is caused by a negative energy balance during first lactation, which negatively affects follicle development and oocyte competence, resulting in lower ovulation rates, impaired embryo development and increased embryo mortality. This may subsequently reduce farrowing rates and litter sizes.

Allowing the sow some time to recover after weaning by inseminating at second estrus after weaning (skip-a-heat), improves reproductive performance. During the recovery period the metabolic state of the sow improves and levels of glucose, insulin and leptin are restored, which results in resumption of normal GnRH release and thus LH (pulsatile) and FSH release to stimulate follicle development. It also directly improves follicle and oocyte quality. Providing a shorter recovery period after weaning, by daily administration of altrenogest, a progesterone analogue, has also been found (in some studies) to improve reproductive performance (ovulation rate, early embryonic development, fetal development, farrowing rates and litter size (a complete overview is given in Chapter 1). This is thought to be related to improved follicle development and follicle quality during treatment. The aim of this thesis was to develop a better understanding of the use of altrenogest after weaning, as a strategy to overcome the negative effects of first lactation on follicle development and subsequent reproductive performance.

Effect of post weaning altrenogest treatments on follicle sizes and embryo development

To study follicle development during and after different post weaning altrenogest treatments and relate this to subsequent fertility, an experiment was carried out (Chapter 2). Primiparous sows (n = 47) were assigned to control, RU8-15 (15 mg altrenogest, for 8 days), RU8-20 (20 mg altrenogest for 8 days) and RU15-15 (15 mg altrenogest for 15 days). All altrenogest treatments started the day before weaning (D-1) and lactation length was on average 21 days. Daily ultrasound revealed a progressive increase in follicle size during altrenogest treatment for the first five days of treatment, after which a plateau was reached at Day 6. The increase in follicle size resulted in larger follicles at the start of the follicular phase for altrenogest treated sows (4.8-4.9 mm) compared to controls (2.9 mm); the size was not affected by dose or duration of treatment. This difference in follicle size remained significant till day 4 of the follicular phase. Onset of estrus was sooner in RU8-15 and RU8-20 then in control animals ($P = 0.005$), but no difference in ovulation rate, CL size or embryo development was found. Ovulation rate, however, was positively related to follicle growth during altrenogest treatment ($b = 1.11$; $P = 0.05$). This study showed, that follicle size increases during altrenogest treatment (independent of dose and duration) resulting in larger follicles at the start of the follicular phase. This did not result in improved fertility on day 5 after ovulation and it was thought that 5 days after ovulation may have been too early to see differences in fertility between treatments.

Effect of post weaning altrenogest treatments on follicle size and litter size

To study the effect of length of treatment on follicle development and subsequent reproductive performance (farrowing rate and litter size) a second study was carried out (Chapter 3). Primiparous sows (n = 259) were assigned to control, RU4 (20 mg altrenogest for 4 days), RU8 (20 mg altrenogest for 8 days) or RU15 (20 mg altrenogest for 15 days). All altrenogest treatments started the day before weaning (= D-1) and lactation length averaged 20 days. Follicle size was similar between treatments at weaning and increased during altrenogest treatment, resulting in larger follicles at the start of the follicular phase (5.3 ± 0.9, 5.5 ± 1.3, 5.1 ± 1.2 *vs.* 3.4 ± 0.6 mm for RU4, RU8, RU15 and controls, respectively; $P < 0.05$). Farrowing rate averaged 87% and was higher in RU15 (95%) than in RU8 (76%; $P = 0.04$). Litter size (Total born) was larger in RU15 than in all other treatments (14.4 ± 3 *vs.* 11.9 ± 3.5, 12.1 ± 3.0 and 12.0 ± 4.3 piglets, for RU15, control, RU4 and RU8, respectively; $P < 0.05$). Farrowing rates were higher for sows with small or medium follicles, than for sows with large follicles at weaning (86% and 78% *vs.* 48%, respectively; $P < 0.001$). Especially in RU8 sows, a large decrease in farrowing rate was seen for sows with large follicles at weaning, compared to sows with small or medium follicles (22% *vs.* 94% and 83%, respectively; $P < 0.05$). The results of this experiment demonstrated that follicle size increases during altrenogest treatment and that a 15 day treatment resulted in improved fertility. It also showed that shorter treatment may only be beneficial for sows with compromised follicular development at weaning and may negatively affect fertility in sows with large follicles at weaning.

Altrenogest before and after weaning in relation to follicle development and fertility

Chapter 3 showed that follicle size at initiation of treatment may influence subsequent fertility and that a large follicle size at weaning may have negative effects on subsequent fertility. Therefore in this study an attempt was made to prevent outgrowth of follicles before weaning, using altrenogest treatment from 3 days before weaning onwards (Chapter 4). Sows (87 parity 1 and 130 parity 2-3) were randomly assigned to one of the following 4 treatments: control, RU0-20 (20 mg altrenogest from D-1 till D6), RU40-20 (40 mg altrenogest from D-3 till D0 + 20 mg of altrenogest from D1 till D6) or RU20-20 (20 mg altrenogest from D-1 till D6). Weaning was at day 21 of lactation = D0. Despite the use of altrenogest before weaning follicle size increased from D-3 till D0 in all treatments. During subsequent altrenogest treatment follicle size increased even further, resulting in larger follicles for altrenogest treated animals than control animals at the start of the follicular phase (5.4 ± 0.1 *vs.* 3.8 ± 0.2 mm, respectively; $P < 0.0001$). Primiparous altrenogest treated sows had larger litters (Total born + mummified fetuses) than control sows (13.4 ± 0.5 *vs.* 11.9 ± 0.7 piglets, respectively; $P = 0.02$). Also in primiparous control sows a positive relation was found between back fat depth at weaning and litter size (total born; slope of the regression line = 0.82; $P < 0.05$), which was absent in altrenogest treated sows. So clearly, the use of altrenogest before weaning did not suppress follicular growth before weaning and no differences between altrenogest treatments in subsequent follicle development and fertility were found. The use of post weaning altrenogest treatment in primiparous sows increased the number of fetuses, but uterine capacity may have been limiting. Especially primiparous sows with

compromised body condition (low back fat depth at weaning) may benefit from post weaning altrenogest treatment, since low back fat depth at weaning was associated with smaller litter sizes in control sows but not in altrenogest treated sows. This may be related to the positive effect of a recovery period after weaning in altrenogest treated sows.

Split weaning before altrenogest treatment and subsequent follicle growth and fertility

To investigate in more detail the effect of follicle size at initiation of treatment on subsequent fertility, as found in chapter 3 in this study, an attempt was made to stimulate follicle growth before weaning to induce differences in follicle size at weaning (Chapter 5). Multiparous sows (n = 47) were either split-weaned (SW; litter size reduced to the smallest 6 piglets 3 days before weaning; n = 23) or normally weaned (C; litter size not reduced; n = 24). All sows were fed altrenogest (20 mg) from the day before complete weaning (weaning at day 24 of lactation = D0) till D5. At weaning no difference in follicle size existed between control or split-weaned sows, but SW sows had larger follicles on D1 and D2 than C sows ($P \leq 0.05$). No differences were found in interval to estrus, ovulation rate or progesterone levels, but C sows had higher total number of embryos and higher embryo survival rates on day 10 of pregnancy than SW sows (25.1 ± 6.6 *vs.* 17.6 ± 9.8 embryos, respectively; $P = 0.005$ and 83 ± 19 *vs.* 58 ± 31%, respectively; $P = 0.001$). When SW sows were divided in sows with low (<63%; n = 10) or high (≥ 63%; n = 10) embryo survival, sows with low embryo survival rate had larger follicles on D3-6 ($P \leq 0.04$). Increase in follicle size during altrenogest feeding was negatively related to embryo survival rate for both C as SW sows ($P < 0.0001$). This study showed that SW sows had reduced embryo survival rates after post weaning altrenogest treatment. Especially SW sows with large follicle sizes and sustained follicle growth during altrenogest feeding suffered from low embryo survival rates. It is possible that the sustained growth and subsequent extended lifespan of the follicles negatively affected follicle quality and oocyte competence and thereby embryo survival. This seems to be confirmed by the observation that sows, that showed a decrease in average follicle size between D5 and D6, had higher embryo survival rates. A decrease in follicle size may indicate that atresia occurred, after which a new "healthy" set of antral follicles was recruited and selected to ovulate ultimately, resulting in improved fertility.

LH pulsatility during the final day of altrenogest treatment and follicle development

The previous studies showed a variation in response to altrenogest treatment but they all showed an increase in follicle size during treatment. Therefore, it was suspected that pulsatile LH release was not completely suppressed during altrenogest treatment. So LH release patterns were investigated during the last day of 15 days post weaning altrenogest treatment in relation to follicle development (Chapter 6). Weaned, catheterized, primiparous sows (n = 10) received 20 mg of altrenogest from D-1 till D13 (weaning at day 26 of lactation = D0). Blood samples were collected from an indwelling jugular vein catheter every 12 minutes from 1000h till 1900h (1st sampling period) and from 2300h till 0800h (2nd sampling period). Altrenogest was administered at 0800h. LH levels were low throughout the first sampling period; only 2 out of 10 sows showed

an LH pulse at the end of the period. During the 2nd sampling period, LH levels increased (average: 0.83 ng/mL (period 2) *vs.* 0.49 ng/mL (period 1) and all sows showed LH pulses (3.1 pulses (period 2) *vs.* 0.33 pulses (period 1). Thus, full suppression of pulsatile LH release was only seen from 3h till 9h after altrenogest administration and by 20h after altrenogest administration all sows had resumed LH pulsatility. Since pulsatile LH release stimulates follicular growth, this variation of suppressive effect of altrenogest on LH release during the 24 hours between 2 altrenogest administrations may explain why generally an increase of follicle size to approximately 5 mm is seen during altrenogest treatment.

Levels of LH, FSH, estradiol and follicle size during altrenogest treatment

The previous study established that LH pulsatility is not fully suppressed during the last day of altrenogest treatment. This study investigated to what extent LH pulsatility was suppressed before and (immediately) after weaning and how FHS and estradiol profiles, and follicle size developed during altrenogest treatment (Chapter 7). Catheterized primiparous sows were assigned to control (n = 11) or altrenogest (20 mg altrenogest from D-1 till D13, weaning at day 26 of lactation = D0; n = 15). Therefore, blood samples were taken every 12 minutes on D-1 and D0 to assess LH levels and every 8 hours during altrenogest treatment to asses FSH and estradiol levels. Fewer altrenogest than control sows showed LH pulses on D-1 ($P = 0.001$). On D0 LH pulsatility was suppressed during the first 4-5 hours after altrenogest feeding in altrenogest sows, but 85% of altrenogest sows showed LH pulses later on. Nevertheless, average and basal LH levels were still lower in altrenogest (average: 0.63 ng/mL and basal: 0.34 ng/mL) than in control sows (average: 0.82 ng/mL and basal: 0.47 ng/mL). Release patterns of FSH and estradiol were related to moment of altrenogest administration with lowest levels 7 hours after administration and highest levels 23 hours after administration. This means that FSH, like LH, is only suppressed for a part of the day, allowing for estradiol production during time when FSH is less suppressed. FSH levels increased from weaning onwards and reached peak levels on D5 after which FSH concentration declined. Coinciding with FSH an increase in follicle size was seen till D5 after which follicle size declined again. Estradiol levels also increased after weaning but peaked already on D2 after which they declined. So follicles showed decreased estrogenic activity after D2 while FSH levels and follicle size increased till D5. So this decrease in estrogenic activity seems to indicate a loss of LH responsiveness. If such follicles are recruited after altrenogest withdrawal, it may explain reduced fertility after intermediate (5-8 days) altrenogest treatments.

Post weaning altrenogest treatment and different application strategies

The experiments clearly showed that follicle growth occurs during altrenogest treatment, most likely as a result of incomplete suppression of (pulsatile) LH release and FSH release. An increase in follicle size to 4-6 mm was seen in all experiments but follicles did not grow out to pre-ovulatory sizes. So LH and FSH levels were high enough to initiate partial recruitment from the follicle pool at weaning, but not high enough, as the suppressive effect of altrenogest became more apparent after some days of treatment, to sustain further outgrowth of the follicles (a more

detailed explanation is given in Chapter 8). A high variation between sows existed in responsiveness to LH, while follicle size was similar, which seemed related to lactational burden. Sows that had lost more bodyweight during lactation showed less responsiveness to LH and thus less estrogenic activity. In order for a post weaning altrenogest treatment to be successful 3 aspects of the application strategy should be taken in consideration: moment of start of treatment, treatment length and dose of treatment. In general good results can be expected after long treatments (12-15 days), because this provides a recovery period that is long enough to restore metabolism and follicle quality. When using short periods of treatment (3-4 days) than no large effects on subsequent fertility are to be expected, because treatment length is not long enough to establish a recovery period after weaning. However, since follicles grow till 5-6 days after weaning under altrenogest treatment, no negative effects are expected of withdrawal, because follicles are still highly responsive to LH and will grow out to pre-ovulatory sizes. It becomes more complicated when using treatment of 5-8 days, because treatment withdrawal occurs when follicles have become (slightly) atretic. Treatment should be initiated before weaning to prevent a large increase in FSH and LH release immediately after weaning and thus prevent rapid growth of follicles after weaning. Large follicles survive longer during periods of low gonadotrophin concentrations and may therefore go into atresia later during altrenogest treatment than smaller follicles. At the moment of withdrawal this results in outgrowth of follicles that are still responsive to LH, but these follicles have aged during altrenogest treatment and will eventually ovulate an oocyte with reduced competence with negative consequences for subsequent reproduction. A similar reduction in fertility may occur in sows that already have large follicles at weaning which are therefore more responsive to LH. No differences between the use of 15 or 20 mg were found, so both dosages maybe used provided that the sows ingest the entire dose. Consequences and practical implications of these findings are further discussed in the general discussion (Chapter 8) of this thesis. In this chapter also recommendations for future research are made.

Samenvatting

Eersteworps zeugen kunnen een verminderde vruchtbaarheid laten zien in de tweede worp, wat zich kenmerkt door een langer interval spenen-bronst, lager afbigpercentage en kleinere tomen. Deze verminderde vruchtbaarheid wordt ook wel het tweedeworpssyndroom genoemd. Het wordt veroorzaakt door een negatieve energie balans tijdens de eerste lactatie wat een negatief effect kan hebben op follikelontwikkeling en eicelcompetentie. Dit resulteert uiteindelijk in lagere ovulatiegraden, verminderde embryo-ontwikkeling, een toename in embryosterfte en dus lagere afbigpercentages en kleinere tomen.

Een herstelperiode na spenen door de zeug pas te insemineren tijdens de tweede bronst na spenen (skip-a-heat) kan de vruchtbaarheid bevorderen. Tijdens deze herstelperiode verbetert de metabole status van de zeug en herstellen glucose, insuline en leptine niveaus wat leidt tot hervatting van normale GnRH afgifte en dus (pulsgewijze) LH en FSH afgifte om de follikelgroei te stimuleren. Ook is er een direct effect van een verbeterde metabole status op follikelontwikkeling en eicelkwaliteit. Een kortere herstelperiode door het dagelijks toedienen van altrenogest, een progesteron analoog, kan in sommige gevallen ook een positief effect op de vruchtbaarheid (ovulatiegraad, vroeg embryonale ontwikkeling, foetale ontwikkeling, afbigpercentage en toomgrootte) hebben (een compleet overzicht wordt gegeven in Hoofdstuk 1). Dit wordt waarschijnlijk veroorzaakt door een verbetering van follikelontwikkeling en follikelkwaliteit tijdens de behandeling. De doelstelling van dit proefschrift was om een beter begrip te krijgen van het gebruik van altrenogest na spenen, om de negatieve effecten van de eerste lactatie op follikelontwikkeling en de hierop volgende vruchtbaarheid te verminderen.

Altrenogest na spenen en gevolgen voor follikelgrootte en embryonale ontwikkeling

Om follikel ontwikkeling tijdens en na verschillende altrenogest behandelingen na spenen te bestuderen en dit te relateren aan hierop volgende vruchtbaarheid is er een experiment uitgevoerd (Hoofdstuk 2). Eersteworps zeugen (n=47) werden ingedeeld als controle, RU8-15 (15 mg altrenogest voor 8 dagen), RU8-20 (20 mg altrenogest voor 8 dagen) en RU15-15 (15 mg altrenogest voor 15 dagen). Alle altrenogest behandelingen begonnen de dag voor spenen (D-1) en lactatielengte was gemiddeld 21 dagen. De zeugen werden dagelijks gescand en follikelgrootte nam toe tijdens de eerste 5 dagen van altrenogest, waarna een plateau in follikelgrootte werd bereikt op Dag 6. Deze toename in follikelgrootte leidde tot grotere follikels bij de start van de follikulaire fase voor altrenogest zeugen (4.8-4.9 mm) in vergelijking met controle zeugen (2.9 mm). Follikelgrootte was niet beïnvloed door dosering of duur van de behandeling. Altrenogest zeugen bleven grotere follikels houden dan controle zeugen tot dag 4 van de follikulaire fase. Het interval tot bronst was korter voor RU8-15 en RU8-20 dan voor controle zeugen ($P = 0.005$), maar ovulatiegraad, CL grootte en embryonale ontwikkeling verschilden niet tussen de behandelingen. Ovulatiegraad was echter wel positief gecorreleerd met follikelgroei tijden altrenogest behandeling ($b = 1.11$; $P = 0.05$). Dit experiment liet zien dat follikelgrootte toeneemt tijdens altrenogest behandeling (onafhankelijk van dosering en duur van behandeling) wat leidt tot grotere follikels

tijdens de start van de follikulaire fase. Dit resulteerde echter niet in verbeterde vruchtbaarheid 5 dagen na ovulatie waarschijnlijk omdat 5 dagen na ovulatie te vroeg was om verschillen tussen de behandelingen te kunnen zien.

Altrenogest na spenen en gevolgen voor follikelgrootte en toomgrootte

Om het effect van duur van de altrenogestbehandeling na spenen op follikelontwikkeling en de hierop volgende vruchtbaarheid (afbigpercentage en toomgrootte) te bestuderen werd een tweede experiment uitgevoerd (Hoofdstuk 3). Eersteworps zeugen (n = 259) werden ingedeeld als controle, RU4 (20 mg altrenogest voor 4 dagen), RU8 (20 mg altrenogest voor 8 dagen) of RU15 (20 mg altrenogest voor 15 dagen). Alle altrenogestbehandelingen startten de dag voor spenen (= D-1) en lactatielengte was gemiddeld 20 dagen. Bij spenen was follikelgrootte vergelijkbaar voor alle behandelingen, maar follikelgrootte nam toe tijdens altrenogestbehandeling wat leidde tot grotere follikels bij het begin van de follikulaire fase (5.3 ± 0.9, 5.5 ± 1.3, 5.1 ± 1.2 *vs.* 3.4 ± 0.6 mm voor respectievelijk, RU4, RU8, RU15 en controle; $P < 0.05$). Afbigpercentage was gemiddeld 87% en was hoger voor RU15 (95%) dan voor RU8 (76%; $P = 0.04$). Toomgrootte (totaal geboren) was hoger voor RU15 dan voor alle andere behandelingen (14.4 ± 3 *vs.* 11.9 ± 3.5, 12.1 ± 3.0 en 12.0 ± 4.3 biggen voor respectievelijk, RU15, controle, RU4 en RU8; $P < 0.05$). Afbigpercentages waren hoger voor zeugen met kleine of medium follikels bij spenen dan voor zeugen met grote follikels bij spenen (respectievelijk, 86% en 78% *vs.* 48%; $P < 0.001$). In het bijzonder was dit het geval in RU8 zeugen; zeugen met grote follikels bij spenen hadden een veel lager afbigpercentage dan zeugen met kleine of medium follikels (respectievelijk, 22% *vs.* 94% en 83%; $P < 0.05$). Uit de resultaten van dit experiment bleek dat follikelgrootte toeneemt tijdens altrenogestbehandeling en dat een behandeling van 15 dagen leidt tot verbeterde vruchtbaarheid. Ook is gebleken dat een kortere behandeling wellicht alleen maar leidt tot verbeterde resultaten in zeugen met kleine follikels bij spenen en zelfs negatieve gevolgen voor vruchtbaarheid kan hebben in zeugen met grote follikels bij spenen.

Altrenogest voor en na spenen en gevolgen voor follikelontwikkeling en vruchtbaarheid

Hoofdstuk 3 liet zien dat follikelgrootte bij het begin van de altrenogestbehandeling de hierop volgende vruchtbaarheid kan beïnvloeden en dat een grote follikelgrootte aan het begin van de behandeling negatieve gevolgen voor vruchtbaarheid kan hebben. Daarom is er in dit experiment geprobeerd om uitgroei van follikels voor spenen te voorkomen door altrenogestbehandelingen 3 dagen voor spenen te beginnen (Hoofdstuk 4). Zeugen (87 eersteworps en 130 tweede -en derdeworps) werden ingedeeld in de volgende 4 behandelingen: controle, RU0-20 (20 mg altrenogest van D-1 tot D6) RU40-20 (40 mg altrenogest van D-3 tot D0 + 20 mg altrenogest van D1 tot D6) of RU20-20 (20 mg altrenogest van D-1 tot D6). Spenen vond plaats op dag 21 van de lactatie (= D0). Ondanks de altrenogestbehandeling voor spenen nam de follikelgrootte toe van D-3 tot D0 in alle behandelingen. Tijdens de hierop volgende altrenogestbehandeling na spenen groeiden de follikels nog verder door wat uiteindelijk resulteerde in grotere follikels aan het begin van de follikulaire fase voor altrenogest zeugen in vergelijking met controle zeugen

(respectievelijk 5.4 ± 0.1 *vs.* 3.8 v 0.2 mm; $P < 0.0001$). Eersteworps, altrenogest zeugen kregen grotere tomen (totaal geboren + mummies) dan controle zeugen (respectievelijk 13.4 ± 0.5 *vs.* 11.9 ± 0.7 biggen; $P = 0.02$). In eersteworps, controle zeugen werd er ook een positieve relatie gevonden tussen spekdikte bij spenen en toomgrootte (totaal geboren; helling van de regressielijn = 0.82; $P < 0.05$), deze relatie werd niet gevonden bij altrenogest zeugen. Altrenogestbehandeling onderdrukte follikelgroei voor spenen niet en er werden geen verschillen gevonden tussen de altrenogestbehandelingen in follikelontwikkeling en vruchtbaarheid. Het gebruik van altrenogest na spenen in eersteworps zeugen resulteerde in een toename van het aantal foetussen, maar mogelijk is de baarmoedercapaciteit een beperkende factor geweest waardoor dit niet resulteerde in meer geboren biggen. In het bijzonder eersteworps zeugen met een lage bodyconditie (weinig spekdikte bij spenen) kunnen mogelijk profiteren van altrenogestbehandeling na spenen omdat weinig spekdikte bij spenen was geassocieerd met kleinere toomgroottes in controle zeugen maar niet in altrenogest zeugen. Dit zou kunnen komen door het positieve effect van een herstelperiode na spenen tijdens behandeling in altrenogest zeugen.

Gedeeltelijk spenen voor altrenogestbehandeling en follikelgrootte en vruchtbaarheid

Om in detail het effect van follikelgrootte bij begin van altrenogestbehandeling op de vruchtbaarheid (zoals in Hoofdstuk 3 werd gevonden) te onderzoeken is er een poging gedaan om follikelgroei voor spenen te stimuleren om zo verschil in follikelgrootte bij spenen te krijgen (Hoofdstuk 5). Meerdereworps zeugen (n = 47) werden of gedeeltelijk gespeend (SW; toomgrootte gereduceerd tot de 6 kleinste biggen 3 dagen voor spenen; n= 23) of normaal gespeend (controle, C; toomgrootte niet gereduceerd voor spenen; n = 24). Alle zeugen kregen altrenogest (20 mg) van de dag voor spenen (spenen vond plaats op dag 24 van lactatie = D0) tot D5. Bij spenen werd er geen verschil in follikelgrootte gevonden tussen controle en gedeeltelijk gespeende zeugen, maar SW zeugen hadden grotere follikels op D1 en D2 dan C zeugen ($P \leq 0.05$). Interval tot bronst, ovulatiegraad en progesteronniveaus verschilden niet tussen C en SW, maar C zeugen hadden meer embryo's en een hogere embryonale overlevingsgraad op dag 10 van de dracht dan SW zeugen (respectievelijk 25.1 ± 6.6 *vs.* 17.6 ± 9.8 embryo's; $P = 0.005$ en 83 ± 19 *vs.* 58 ± 31; $P = 0.001$). Als SW zeugen werden onderverdeeld in zeugen met een lage (<63%; n=10) of een hoge (≥63%; n = 10) embryonale overlevingsgraad dan hadden zeugen met een lage embryonale overlevingsgraad grotere follikels op D3-6 ($P \leq 0.04$). Een toename in follikelgrootte tijdens altrenogest behandeling was negatief gecorreleerd met embryonale overlevingsgraad in zowel C als SW zeugen ($P < 0.0001$). Dit experiment liet zien dat SW zeugen verminderde embryonale overleving hadden na een altrenogest behandeling na spenen. In het bijzonder SW zeugen met grote follikels en continue follikelgroei tijdens altrenogest behandeling hadden lagere embryonale overlevingsgraden. Mogelijkerwijs heeft continue follikelgroei tijdens altrenogest behandeling en daardoor een verlengde levensduur van de follikel een negatief effect op follikelkwaliteit en eicelcompetentie, wat kan leiden tot verminderde embryonale overleving. Dit lijkt bevestigd door het feit dat zeugen, die een daling in follikelgrootte lieten zien tussen D5 en 6, hogere embryonale overlev-

ingsgraden hadden. Een afname in follikelgrootte kan betekenen dat atresie optrad, waarna een "gezonde" groep antrale follikels werd gerekruteerd en uiteindelijk geselecteerd voor ovulatie, wat resulteerde in verbeterde vruchtbaarheid.

LH pulsatiliteit tijdens de laatste dag van altrenogestbehandeling en follikelontwikkeling

De voorgaande experimenten lieten een variabele respons op altrenogestbehandeling zien, maar follikelgroei tijdens altrenogestbehandeling werd waargenomen in alle experimenten. Daarom werd er vermoed dat pulsatiele LH afgifte niet volledig onderdrukt was tijdens altrenogestbehandeling en werd LH afgifte tijdens de laatste dag van een 15-daagse altrenogestbehandeling na spenen onderzocht in relatie tot follikelontwikkeling (Hoofdstuk 6). Gespeende, gecanuleerde, eersteworps zeugen (n = 10) kregen 20 mg altrenogest van D-1 tot D13 (spenen vond plaats op dag 26 van lactatie = D0). Bloedmonsters werden verzameld met behulp van een vene jugularis canule met een interval van 12 minuten van 10.00 uur tot 19.00 uur (1ste monstername) en van 23.00 uur tot 08.00 uur (2e monstername). Altrenogest werd toegediend om 08.00 uur. LH niveaus waren laag gedurende de eerste monstername; slechts 2 van de 10 zeugen lieten aan het eind van de periode een LH puls zien. Tijdens de 2e monstername namen de LH niveaus toe (gemiddeld: 0.83 ng/mL (monstername 2) *vs*. 0.49 ng/mL (monstername 1) en lieten alle zeugen LH pulsen zien (3.1 pulsen (monstername 2) *vs*. 0.33 pulsen (monstername 1). Dus volledige onderdrukking van de LH pulsatiliteit werd alleen waargenomen van 3 tot 9 uur na altrenogesttoediening en 20 uur na altrenogesttoediening was pulsatiele LH afgifte hervat in alle zeugen. Omdat pulsatiele LH afgifte de follikelgroei stimuleert, kan deze variatie in onderdrukking van de LH afgifte tijdens de 24 uur tussen 2 altrenogesttoedieningen verklaren waarom een toename van follikelgrootte tot ongeveer 5 mm algemeen wordt waargenomen tijdens altrenogestbehandeling.

LH, FSH en estradiol niveaus en follikelgrootte tijdens altrenogest behandeling

Uit het vorige experiment bleek dat LH pulsatiliteit niet volledig onderdrukt wordt tijdens de laatste dag van altrenogest behandeling. Dit experiment onderzocht hoe LH pulsatiliteit onderdrukt was voor en (direct) na spenen en hoe FSH en estradiol niveaus en follikelgrootte zich ontwikkelden tijdens altrenogest behandeling (Hoofdstuk 7). Gecanuleerde, eersteworps zeugen werden ingedeeld als controle (n = 11) of altrenogest (20 mg altrenogest van D-1 tot D13, spenen vond plaats op dag 26 van lactatie = D0; n = 15). Bloedmonsters werden genomen op D-1 en D0 met intervallen van 12 minuten om LH niveaus te bepalen en elke 8 uur om FSH en estradiol niveaus te bepalen. Minder altrenogest zeugen dan controle zeugen hadden LH pulsen op D-1 ($P = 0.001$). LH pulsatiliteit op D0 was onderdrukt gedurende de eerste 4-5 uren na altrenogesttoediening, maar 85% van de altrenogest zeugen liet hierna LH pulsen zien. Desalniettemin, waren gemiddelde en basale LH niveaus lager in altrenogest zeugen (gemiddeld: 0.63 ng/mL en basaal: 0.34 ng/mL) dan in controle zeugen (gemiddeld: 0.82 ng/mL en basaal: 0.47 ng/mL). Afgiftepatronen van FSH en estradiol waren gerelateerd aan het moment van altrenogesttoediening en laagste niveaus werden gevonden 7 uur na altrenogesttoedieining en hoogste niveaus werden

gevonden 23 uur na altrenogesttoediening. Dit betekent dat FSH, net zoals LH, alleen voor een gedeelte van de dag onderdrukt is, waardoor er estradiolsecretie optreedt tijdens de periode waarin FSH minder onderdrukt is. FSH niveaus stegen na spenen en bereikten hun hoogtepunt op D5 waarna FSH niveaus weer daalden. Tegelijkertijd werd er ook een toename in follikelgrootte waargenomen tot D5 waarna follikelgrootte afnam. Estradiol niveaus stegen ook na spenen maar bereikten hun hoogtepunt al op D2 waarna ze weer afnamen. Dus follikels hadden verminderde oestrogene activiteit na D2 terwijl FSH niveaus en follikelgrootte stegen tot D5. Het lijkt erop dat deze afname in oestrogene activiteit te maken heeft met een verminderde responsiviteit van de follikels voor LH. Als zulke follikels gerekruteerd worden na het stoppen van de altrenogestbehandeling, zou het de verminderde vruchtbaarheid kunnen verklaren na altrenogestbehandelingen met een duur van 5-8 dagen.

Altrenogest behandeling na spenen en verschillende toepassingsstrategieën.

Uit de experimenten is duidelijk gebleken dat follikelgroei optreedt tijdens altrenogestbehandeling. Waarschijnlijk wordt dit veroorzaakt door onvolledige onderdrukking van (pulsatiele) LH afgifte en FSH afgifte tijdens behandeling. In alle experimenten nam follikelgrootte met ongeveer 4-6 mm toe, maar groeiden follikels niet uit tot pre-ovulatoire grootte. Dus LH en FSH niveaus waren hoog genoeg om een initiële, gedeeltelijke rekrutering van de follikelpopulatie bij spenen op gang te brengen, maar niet hoog genoeg, omdat het onderdrukkende effect van altrenogest sterker werd na een paar dagen van behandeling, om een verdere uitgroei van de follikels te onderhouden (in Hoofdstuk 8 wordt dit verder toegelicht). De responsiviteit op LH varieerde enorm tussen zeugen met vergelijkbare follikelgroottes wat gerelateerd leek te zijn aan lactatiestress. Zeugen die meer gewicht hadden verloren tijdens lactatie bleken minder LH responsiviteit en dus verminderde oestrogene activiteit te vertonen. Om altrenogest na spenen succesvol toe te kunnen passen moet er rekening gehouden worden met de volgende 3 aspecten: moment van het starten van de behandeling, duur van de behandeling en dosering van de behandeling. Over het algemeen kunnen goede vruchtbaarheidsresultaten verwacht worden na lange behandelingen (12-15 dagen), omdat deze herstelperiode lang genoeg is om metabolisme en follikelkwaliteit te herstellen. Bij korte behandelingen (3-4 dagen) hoeven geen grote effecten op vruchtbaarheid te worden verwacht, omdat duur van de behandeling niet lang genoeg is om te herstellen na spenen. Maar omdat follikels tot 5-6 dagen na spenen blijven groeien onder altrenogestbehandeling zijn er geen negatieve effecten van korte behandeling met altrenogest te verwachten, want de follikels zijn op het moment van stoppen van de behandeling nog steeds gevoelig voor stimulatie door LH en zullen dus uitgroeien tot pre-ovulatoire follikels. Dit is ingewikkelder bij behandelingen van 5-8 dagen omdat het stoppen van de behandeling dan samenvalt met het (gedeeltelijk) in atresie gaan van de follikels. Om een stijging van de FSH en LH niveaus na spenen en dus een snelle uitgroei van de follikels na spenen te voorkomen zou de altrenogestbehandeling voor spenen moeten beginnen. Grotere follikels kunnen langer overleven met minder gonadotrope stimulatie en gaan daardoor mogelijk later in atresie tijdens altrenogest behandeling dan kleine follikels. Op het moment van stoppen van de behandeling kan dit leidden tot uitgroei van follikels

die nog steeds gevoelig zijn voor LH stimulatie, maar omdat deze follikels verouderd zijn tijdens altrenogest behandeling kan de eicelcompetentie van deze follikels zijn verminderd waardoor vruchtbaarheid negatief kan worden beïnvloed. Een vergelijkbare afname in vruchtbaarheid kan optreden bij zeugen die al grote follikels (en daardoor meer LH responsiviteit vertoonden) hadden op het moment van spenen. Er zijn geen verschillen gevonden in behandelen met 15 of met 20 mg, dus het mag aangenomen worden dat beide doseringen gebruikt kunnen worden zolang de volledige dosis opgenomen word. Consequenties en praktische implicaties van deze bevindingen worden verder besproken in de algemene discussie (Hoofdstuk 8) van dit proefschrift. In dit hoofdstuk worden ook enkele aanbevelingen voor verder onderzoek gedaan.

Curriculum Vitae

Jessika Judith Johanna van Leeuwen was born September 10, 1981 in Harderwijk, the Netherlands, where she spent the first 11 years of her childhood. In 1992 she moved with her family to Dalfsen.

She graduated from high school (Thomas a Kempis College, Zwolle) in 1999 and started her career in Animal Sciences at Wageningen University the same year. She specialized herself in Animal Behavior and Reproductive Physiology. She investigated the behavior of captive bottlenose dolphins during sessions of "Dolphin Assisted Therapy" at Dolfinarium Harderwijk and feeding behavior of weaned piglets to optimize feeding strategies at the Swine Research Centre of Nutreco. To specialize in dairy fertility she went to the University of Florida where she worked in the lab of Dr. Thatcher on optimizing resynchronization of estrus in dairy cows. She graduated in 2004 for her MSc after which she started to work as a project coordinator for the Product Boards of Horticulture, Livestock Meat and Eggs and Arable Farming in the Netherlands.

In February 2007 she started in Argentina with the field work for her PhD at the Adaptation Physiology Group of Wageningen University. The results of this work are described in this thesis. In May 2011 she started to work for IMV-Technologies as Scientific and Technical Manager in the R&D Department. Jessika is married to Juan Pablo Ibarrola and lives with him in l'Aigle, France.

Publications

Refereed Scientific Journals

Bartolome J. A., van Leeuwen J. J. J., Thieme M., Sa'filho O.G., Melendez P., Archbald L. F., Thatcher W. W., 2009. Synchronization and resynchronization of inseminations in lactating dairy cows with the CIDR insert and the Ovsynch protocol. Theriogenology 72(6):869-78.

Leeuwen van J.J.J., Williams S.I., Kemp B., Soede N.M., 2010. Post-weaning Altrenogest treatment in primiparous sows; the effect of duration and dosage on follicular development and consequences for early pregnancy. Anim. Repro. Sci.119: 258-264

Leeuwen van J.J.J., Williams S.I., Martens M.R.T.M., Jourquin J., Driancourt M.A., Kemp B., Soede N.M., 2011. The effect of different post-weaning altrenogest treatments of primiparous sows on follicular development, pregnancy rates and litter sizes. J. Anim. Sci. 89: 397-403.

Leeuwen van J.J.J., Martens M.R.T.M., Jourquin J., Driancourt M.A., Kemp B., Soede N.M., 2011. Variation in LH pulsatility during 24 hours after a postweaning altrenogest treatment in relation to follicle development in primiparous sows. Anim. Reprod. Sci. 126: 101–107.

Leeuwen van J.J.J., Martens M.R.T.M., Jourquin J., Driancourt M.A., Kemp B., Soede N.M., 2011. Effects of altrenogest treatments before and after weaning on follicular development, farrowing rate, and litter size in sows J. Anim Sci. 89: 2397-2406.

Leeuwen van J.J.J., Verhoeven M.T.W., van der Heden- van Noort I.M., Kemp B., Soede N.M., Split weaning before altrenogest synchronization of multiparous sows alters follicular development and reduces embryo survival. *Accepted by Reprod. Dom. Anim.*

Conference Proceedings and Abstracts

Leeuwen, J.J.J. van, Williams, S. , Kemp, B., Soede, N.M. 2009. Post-weaning Altrenogest treatment in primiparous sows: the effect of duration and dosage on follicular development and consequences for early pregnancy. In Proceedings of the VIII International Conference on Pig Reproduction, Banff, Alberta, Canada, 31 May - 4 June 2009 (pp. 95-96).

Leeuwen, J.J.J. van, Williams, S., Kemp, B., Soede, N.M. 2009. Post-weaning Altrenogest treatment in primiparous sows; the effect of duration and dosage on follicular development and consequences for early pregnancy. Society of Reproduction and Fertility supplement 66: 197 -198.

Other publications

de Vries A., van Leeuwen J., Thatcher W., *Economic importance of improved reproductive performance*. 2004. Proceedings of the reproduction road show. University of Florida / IFAS extension

Kemp, B., Wientjes, J.G.M., Leeuwen, J.J.J., Hoving, L.L., Soede, N.M., 2011. Nutrition and management during lactation: effects on future parity productivity. In Proceedings of Manitoba Swine Seminar 2011 volume 25. Sharing ideas and information for efficient pork production. Manitoba, Canada, 2-3 February 2011 (pp 85-97).

Training and Supervision plan

	year
The Basic Package (3.0 ECTS)	
WIAS Introduction Course	2007
Course on philosophy of science and/or ethics	2008
International conferences (4.0 ECTS)	
3rd International Symposium on swine production, Aguas de Lindoia, Brasil	2007
ICAR 2008, Budapest, Hungary	2008
INITRA UBA 2008, Buenos Aires, Argentina	2008
International Conference on Pig Reproduction (ICPR), Banff, Canada	2009
G.I.T.E.P. meeting, Rosario, Argentina	2009
ESDAR, Eger, Hungary	2010
Seminars and workshops (1.2 ECTS)	
WIAS Science Day, Wageningen, the Netherlands	2009
G.I.T.E.P, 2009, Gral Belgrano, Argentina	2009
WIAS Seminar on Lactation Management, Wageningen, the Netherlands	2009
WIAS Reproduction Seminar, Wageningen, the Netherlands	2010
Presentations (7.0 ECTS)	
Jornada de Ciencia UNLP; Oral Presentation, Gral Pico, Argentina	2007
Jornadas Internacionales INITRA, Poster, Buenos Aires, Argentina	2008
WIAS Science day, poster, Wageningen, the Netherlands	2009
ICPR Oral Presentation, Banff Canada,	2009
ICPR, Poster, Banff, Canada	2009
G.I.T.E.P. Oral Presentation, Gral Belgrano, Argentina	2009
WIAS Reproduction Seminar, Wageningen, the Netherlands	2010
In-Depth Studies (6.0 ECTS)	
Inseminación artificial y ecografía en porcinos, UNLP, Argentina	2007
Actualización de reproducción en animales domésticos, UNLP, Argentina	2010
Actualización sobre enfermedades emergentes y re-emergentes del cerdo, UNLP, Argentina	2010
WIAS Advanced Statistics Course	2007
Generalized Linear Models	2009

	year
Professional Skills Support Courses (4.2 ECTS)	
Communication and Interdisciplinary Research	2009
Scientific Writing	2010
Career Assessment	2010
Effective Behaviour in your Professional Surroundings	2010
Research Skills Training (6.0 ECTS)	
Preparing own PhD research proposal	2007
Didactic Skills Training (13.1 ECTS)	
Lectures Cell Biology, UNLP, Argentina	2007
Practicum ultrasound in swine, bovine and ovine, UNLP, Argentina	2007-2008
Reproductive Anatomy Swine Practicum, Cordoba, Argentina	2008
Practicum ADP2	2009-2010
Supervising 6 MSc students	2007-2011
Inleiding Dierwetenschappen	2010
Education and Training	**Total 44.5 ECTS**

Colophon

This thesis is printed by GVO drukker | Ponsen & Looyen B.V., Ede, the Netherlands.

Artwork and Layout of the cover by Anniek van Leeuwen (Nico M. Productions)

This research and thesis were funded by:

Intervet Schering Plough Animal Health, Boxmeer, the Netherlands & Janssen Animal Health, Beerse, Belgium

MSD Animal Health

JANSSEN ANIMAL HEALTH
a division of
Janssen Pharmaceutica NV

This thesis was sponsored by:

imv TECHNOLOGIES