Cystic ovarian disease in dairy cattle

Treatment, heritability, and epidemiology

Cysteus ovariële follikels bij melkkoeien

Behandeling, erfelijkheid en epidemiologie

(met een samenvatting in het Nederlands)

PROEFSCHRIFT

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Chapter 1

General introduction

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Introduction

Cystic ovarian disease (COD) in dairy cattle is generally defined as the presence of one or more ovarian follicular structures of at least 2.5 cm in diameter that persist on the ovary for at least 10 days in the absence of a *corpus luteum* (Roberts, 1971; Kesler and Garverick, 1982; Lopez-Diaz and Bosu, 1992). There is no consensus about cyst diameter, although many authors use a diameter of 2.0 cm as a minimum (Arbeiter *et al*., 1990; Cook *et al*., 1991; Boryczko *et al*., 1995; Fleischer *et al*., 2001). Also the cyst may present for less than 10 days (Arbeiter *et al*., 1990). Retrospective field studies tend not to investigate the duration of the cyst, because under field conditions a COD diagnosis is mostly made after a single clinical examination and is often followed by immediate treatment of the cyst(s). Cysts have a variable lifespan and are sometimes reported to occur together with a *corpus luteum* (Farin and Estill, 1993). However, the absence of a *corpus luteum* is an essential criterion of COD in dairy cows (Arbeiter *et al*., 1990).

In this thesis the results of longitudinal field studies are described, focused on the treatment of COD with gonadotrophin-releasing hormone (GnRH), the heritability of COD and correlations between COD and milk production traits, and the relationship between a negative energy balance (NEB) and COD. Data were obtained by staff from one veterinary practice[1] during regular herd monitoring visits. The fertility examination protocol used is extensively described in section 5.2.

In this chapter current knowledge of the above-mentioned aspects of COD is reviewed, and attention is paid to Polycystic Ovarian Syndrome (PCOS) in women, which shows differences, but also similarities, with COD in cattle (section 4). The objectives of our studies are described in more detail in section 5.3.

1.1. Lactational incidence risk and prevalence

The incidence of COD in dairy cows varies between 5% and 10%, although rates as high as 30% have been reported (Casida and Chapman, 1951; Kesler and Garverick, 1982; Laporte *et al*., 1994; Fleischer *et al*., 2001). Yet other studies failed to define the incidence and/or the prevalence (Lopez-Diaz and Bosu, 1992). The incidence describes the number of new cases that arise in a given population over a specified period of time (Thrusfield, 1995), whereas the prevalence represents the fraction of existing cases in a population at a certain moment and depends on how many new cases arise per time unit

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and on the duration of the disease (Thrusfield, 1995). Because it is not relevant to determine the number of COD cases on a certain day of lactation, the prevalence of COD appears not to be a useful parameter. This is clearly shown in a study of Heuer *et al*. (1999). The interquartile range (10-90%) of the occurrence of COD *post partum* was between 33 days and 148 days (n=1152). In another study, 24% of the cysts that appeared before 39 days *post partum* receded spontaneously (Arbeiter *et al*., 1990). Youngquist (1986) stated that spontaneous recovery might reach 60% if the condition is present before 30 days *post partum*. Thus, the estimated incidence is influenced by when the first examination *post partum* takes place and thus depends on the fertility protocol used (Opsomer *et al*., 1996). The incidence of COD also depends on parity. In studies by Hackett and Batra (1985, n=1830) and Fleischer *et al*. (2001, n=2197) the incidence of COD in the lactational period was 5.7% and 7.4% in heifers, respectively, and 18% and 13.7% in multiparous cows, respectively.

1.2. Clinical signs

Clinical signs of COD vary and depend on the extent of luteinization of the cyst. In most cases (62-85%), cows with cysts are anoestrous (Day, 1991; Watson and Cliff, 1997) as a result of the production of progesterone by luteinized cysts. Nymphomania and irregular cycles are also common (Kasari *et al*., 1996). In their study, Watson and Cliff (1997) detected COD during investigations for anoestrus (58%) and irregular heat (12%), at a negative pregnancy diagnosis (12%), and at a pre-breeding examination (17%). Opsomer *et al*. (1998), in their study of ovarian dysfunction in 335 high-yielding dairy cows, found cystic structures in 6% of cows showing anoestrus or delayed cyclicity. It is difficult to distinguish between follicular and luteinized cysts without using ultrasonography in anoestrous cows (Jeffcoate and Ayliffe, 1995), but this distinction is not important in practice, because the response of both types of cysts to GnRH treatment is similar (Youngquist, 1986; Dinsmore *et al*., 1989; Day, 1991). COD always results in an aberrant reproductive performance, leading to extended calving intervals.

1.3. Diagnosis

Rectal palpation, ultrasonography, and determination of progesterone concentrations in milk or plasma are common diagnostic tools to diagnose COD. For a long time, rectal palpation was the only way to diagnose COD. Measurement of progesterone in plasma and milk samples was introduced 30 years ago (Robertson, 1972; Lamming and Bulman, 1976). Real-time B-mode ultrasonography was introduced to veterinary practice in the early 1980s and offered a new tool for early pregnancy diagnosis (Taverne, 1984). It is nowadays used for different species, not only to assess pathological or physiological structures of the genital tract, but also to detect pregnancy (Kähn, 1991). The assessment of bovine ovarian structures by ultrasonography developed further in the last 15 years (Pierson and Ginther, 1984; Sirois and Fortune, 1988; Pieterse, 1989; Kähn, 1991; Garcia *et al*., 1999; Hanzen *et al.,* 2000).

We did not find studies of the sensitivity and specificity of rectal palpation regardless of the type of cyst. Moreover, it has been demonstrated that cysts may persist, regress, or be replaced by others (Cook *et al*., 1990). In their study of the use of milk progesterone analysis for examining postpartal ovarian activity, Opsomer *et al*. (1999b) stated that several so-called cystic structures are normally functioning *corpora lutea*. The accuracy of manual palpation or ultrasonography can be increased by obtaining information about the reproductive history of the animal and by palpation of the uterine horns, vaginal examination, or progesterone determination (Hanzen *et al*., 2000).

1.4. Aetiology of COD

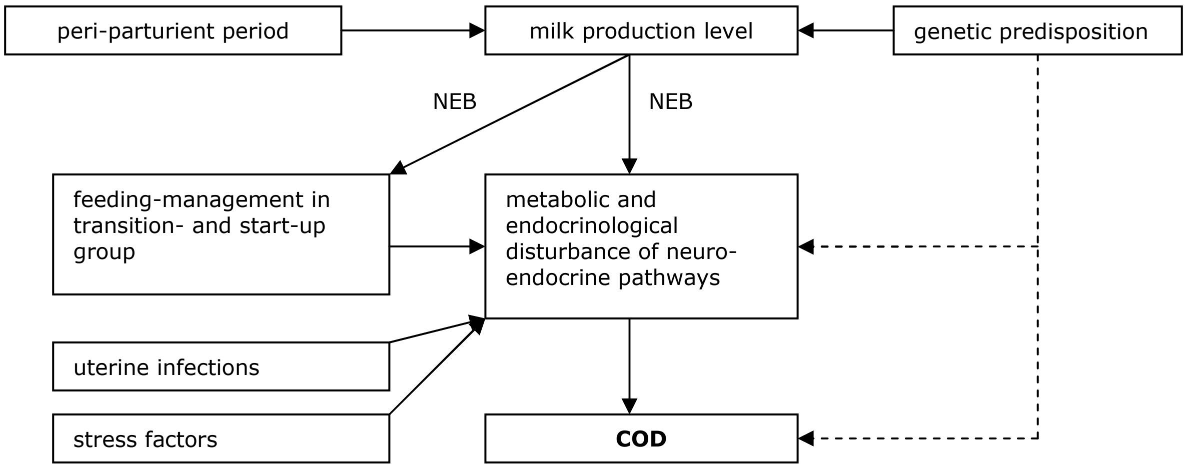
1.4.1. Patho-physiological aspects

The pulsatile nature of GnRH secretion, resulting in pulsatile secretions of luteinizing hormone (LH), is the most important individual signal controlling the activity of the reproductive system (Thiéry and Martin, 1991). GnRH is a decapeptide, synthesized by neurons in the tonic and surge GnRH centre (Senger, 1999) in the hypothalamus. GnRH, in short, stimulates the release of the gonadotrophic glycoproteins LH and follicle-stimulating hormone (FSH) from the anterior pituitary gland. These gonadotrophins stimulate the ovary to secrete oestradiol, which exerts a positive feedback on the neurons of the hypothalamic surge centre, leading to the pre-ovulatory LH surge (Senger, 1999).

Many studies have investigated the aetiology of COD (Figure 1). In general, authors agree that COD is the ultimate result of a neuroendocrine imbalance of the hypothalamo-pituitary-gonadal axis (Lopez-Diaz and Bosu, 1992). Such an imbalance may be provoked by a negative energy balance (NEB) in the early postpartum period (Britt, 1992; Beam and Butler, 1999). While the level of milk production of cows is predominantly genetically determined, phenotypic aspects, such as feeding management in the peri-parturient period, determine the extent to which metabolic and endocrinological disturbances occur.

However, the factors and the way in which they influence the development of COD are not fully understood.

FIGURE 1. A simplified overview of the subjects of study of the pathogenesis of cystic ovarian disease. NEB = negative energy balance.



Energy balance is defined as the difference between net energy intake and net energy requirement (Heuer, 2000). In the context of the postpartum cow, energy is expended on body maintenance and milk production (Beam and Butler, 1999) and can be quantified by using measures of milk production, dietary intake, and body weight (Spicer *et al*., 1990). In the early postpartum period, cows have a higher energy requirement than can be supported by dietary intake and therefore are in NEB for a period (Bauman and Currie, 1980; Canfield and Butler, 1991). In a large-scale field trial, it is impossible to measure the quantity and quality of total dietary intake and therefore, proxy parameters have been developed to determine NEB (Heuer, 2000). The disadvantage of these proxy parameters is their inaccuracy with respect to the real NEB status, which differs per parameter.

Negative energy balance is negatively associated with reproductive function *post partum* (Butler and Smith, 1989). A delayed NEB nadir probably results in a low LH pulse frequency (Beam and Butler, 1999). Insulin, insulin-like growth factors (IGF-I, IGF-II), and insulin-like growth factor-binding proteins (IGFBPs) seem to be metabolic modulators of ovarian activity during NEB (Spicer *et al*., 1990; Spicer and Echternkamp, 1995) in different species. Leptin, a protein hormone, is secreted almost exclusively by adipocytes (Bell and Bauman, 1997) and is thought to be a metabolic signal to the neuroendocrine reproductive system. When energy reserves are inadequate, low leptin levels may inhibit the activity of the neuroendrocine reproductive axis. Studies of mice, rats, and monkeys have shown that, by modulating the expression of its own receptor, leptin regulates neurons containing hypothalamic peptides such as proopiomelanocortin (POMC) and neuropeptide-Y (NPY). Changes in the synthesis and/or release of these or other hypothalamic peptides could then act on GnRH neurons to affect GnRH release (Cunningham *et al.,* 1999). In a study by Block *et al*. (2001) with dairy cows, plasma leptin concentrations were negatively correlated with NEB, growth hormone (GH), and non-esterified fatty acids (NEFAs), and positively correlated with plasma concentrations of insulin and glucose. Villa-Godoy *et al*. (1988) observed that the mean energy balance during postpartum anovulation in cattle was positively correlated with luteal function in the second and third oestrous cycles. Overall, a NEB has an adverse effect on luteal function (Spicer *et al*., 1990).

Uterine infections *post partum* may provoke increased secretion of prostaglandin $F_{2\alpha}$ and cortisol, which are associated with the development of COD (Etherington *et al*., 1985a; Etherington *et al*., 1985b; Bosu and Peter, 1987). Ribadu *et al*. (2000) suggested an interaction between LH pulse frequency and high oestradiol concentrations following ACTH treatment in inducing cyst formation in heifers. Thus, factors that cause stress in dairy cows may also play a role in the development of COD. Studies into a possible genetic predisposition of dairy cows for COD will be discussed later on.

1.4.2. Epidemiological aspects

Many studies have investigated the relation between (proxy parameters of) NEB and resumption of ovarian cyclicity or reproductive performance after calving, but relatively few reports are available about the relation between NEB and COD. In a study by Sovani *et al*. (2000), NEB nadir in the first week after calving as well as the cumulative NEB (NEB area under the curve of lactation week 1-12) predisposed cows to COD.

Body condition and COD have been investigated in some studies. Laporte *et al*. (1994) stated that, in general, COD is a response of the cow to over-consumption of body energy reserves. Gearhart *et al*. (1990) concluded that cows that were overconditioned at drying off were more likely to develop reproductive problems such as COD, because a diminished periparturient intake of dry matter results in NEB and fatty liver disease (Rukkwamsuk *et al*., 1999; Bertrics *et al*., 1992; Jorritsma *et al*., 2000). However, Waltner *et al*. (1993) did not find a correlation between body condition score and the incidence of COD in a herd with an average production of 9541 kg fat corrected milk (FCM). Jorritsma *et al*. (2000) concluded that COD was not related to higher levels of triacylglycerols in the liver (fatty liver) after calving.

Cows with raised levels of milk ketones had a higher risk of having cystic ovaries diagnosed in the following 4 days than did non-ketotic cows (Dohoo and Martin, 1984). Andersson *et al*. (1991) reported an increased risk of COD (OR=8.7) in first-lactating heifers with acetone concentrations > 2.00 mM, but these authors did not indicate the frequency distribution of when COD was diagnosed. This is of importance because some cows with COD re-establish ovarian cyclicity spontaneously (Steinbauer, 1985; Arbeiter *et al*., 1990; Lopez-Diaz and Bosu, 1992).

Heuer *et al*. (1999) concluded, in a study of Dutch dairy herds, that cows with a fat/protein ratio > 1.5 at the first milk recording *post partum,* which is a proxy parameter for NEB, had an increased risk of COD (OR=1.7). However, in a study of a Fleckvieh herd, no significant relationship was found between the fat/protein ratio at the first milk recording *post partum* and the incidence of COD (Heuer and Plug, 1994). The discrepancy between these studies might be explained by the different breeds investigated.

COD has been correlated to milk yield in the concurrent lactation (Erb *et al*., 1981; Erb *et al*., 1985; Gröhn *et al*., 1990; Laporte *et al*., 1994; Heuer *et al*., 1999); however, Booth (1988) and Nanda *et al*. (1989) failed to find such a positive correlation between milk yield and COD. Earlier, Kesler and Garverick (1982) suggested that increased milk production might be the result, rather than the cause, of a hormonal imbalance in cows with COD. However, in dairy practice, an increased milk yield tends to be accompanied by COD (Dobbelaar, 2003).

It should be borne in mind that the different outcomes of studies of the relation of NEB and COD may be due to the (limited) sensitivity and specificity of the proxy parameters of NEB, such as body condition score, elevated levels of ketones, and the fat/protein ratio at the first milk recording *post partum.* The decrease in condition score, rather than the absolute value, may show a better correlation with NEB.

Factors influencing the development of COD are still not fully understood. A major problem is that most studies measured hormone levels only after the condition had been diagnosed (Eyestone and Ax, 1984). Moreover, significant correlations do not necessarily indicate causal relationships (Fleischer *et al*., 2001).

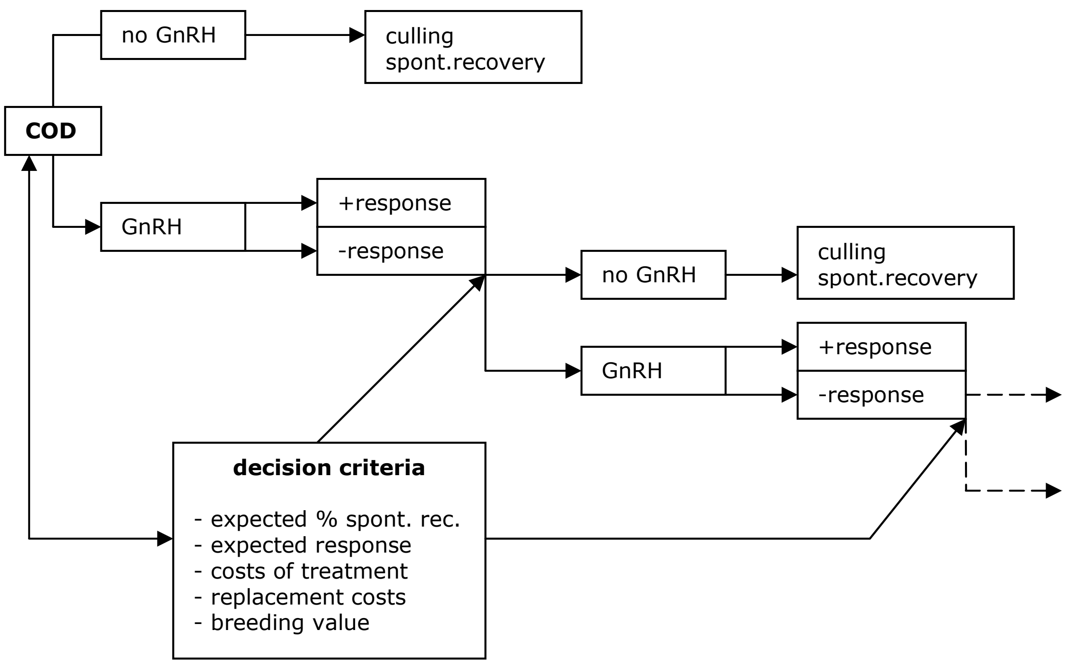
1.5. Economic impact

The time of diagnosis *post partum* and the results of treatment mainly determine the economic impact of COD by influencing the interval between calving and conception. It is not possible to calculate the economic impact of COD on dairy farms in general, because each farm has its own herd-specific variables (e.g. production level, milk price, feeding management, feeding costs, genetic make-up, culling rate). However, dynamic probabilistic simulation makes it possible to calculate the optimal calving pattern (Jalvingh *et al*., 1994) for each farm. Thus, in order to compare studies, insight is needed into specific husbandry conditions, the moment of diagnosis *post partum*, the frequency of visits according to the fertility protocol, the definition of COD used, and the diagnostic tools applied.

2. Treatment of COD

Treatment of COD will be discussed from a clinical and an economic point of view. Both are determined by the timing of diagnosis and the expected spontaneous recovery (Seguin *et al*., 1976; White and Erb, 1980; Steinbauer, 1985; Youngquist, 1986; Dinsmore *et al*., 1989). Clinically, treatment of COD concerns the re-establishment of ovarian cyclicity with subsequent insemination and pregnancy. Economically, the decision

FIGURE 2. Decision tree approach to treatment of cystic ovarian disease (COD) (Adapted from Scholl *et al*., 1992).



to treat an animal is influenced by the costs and the expected benefits of treatment of COD, the costs of replacement, and the breeding value of the cow (Bierschwal *et al.,* 1975; Dinsmore *et al*., 1989; Scholl *et al*., 1992; Suriyasathaporn *et al*., 1998; Jou *et al*., 1999; Douthwaite and Dobson, 2000). It is possible to develop a farm specific decision tree approach for the treatment of COD (Figure 2). The results of various studies will not be discussed extensively here, because of differences in study design, treatment doses, parameters, moment of treatment after calving, and definition of clinical response.

2.1. Gonadotrophin-Releasing Hormone (GnRH)

Treatment of cows with COD with GnRH induces the release of LH, with a maximum plasma LH concentration being reached 90 to 150 minutes after application (Seguin *et al*., 1976; Kruip *et al*., 1977). LH release initiates the formation of active luteal tissue, as indicated by increased serum progesterone levels 7 days after treatment onwards (Seguin *et al.,* 1976; Kruip *et al*., 1977). GnRH can be used for both follicular and luteal cysts (Youngquist, 1986) and usually results in luteinization of the cysts followed by oestrus within 4 weeks of treatment (Dinsmore *et al*., 1990). Dinsmore *et al*. (1987) concluded in a comparative study that there was no difference in clinical response and fertility results after treatment with buserilin acetate or gonadorelin acetate. The dosage of GnRH used varies between 50 to 500 µg depending on the manufacturer's advice. Seguin *et al*. (1976) demonstrated that the serum progesterone levels increased by more than 2 ng/ml on day 11 after treatment with 50, 100, 150, and 250 µg GnRH.

2.2. Other therapies of COD

Like GnRH, human Chorionic Gonadotrophin (hCG) has LH activity, resulting in luteinization of the cyst. GnRH and hCG elicit equivalent endocrine and clinical responses, but GnRH has an advantage over hCG in its minimal antigenicity (Drost and Thatcher, 1992).

Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) has also been used because of its luteolytic activity, and oestrous symptoms can be observed within 2 or 3 days of treatment (Kesler and Garverick, 1982). Leslie and Bosu (1983) found, in a study of 62 cows with luteal cysts, a conception rate of 87.5% (21/24) following artificial insemination during the oestrus induced by the $PGF_{2\alpha}$ analogue fenprostalene. Dinsmore *et al*. (1990) stated that treatment of ovarian cysts with prostaglandins, either alone or in combination with GnRH, was not consistently more effective than with GnRH alone.

Application of progesterone (ear-implant, intra-vaginal device) for 9-12 days with simultaneous administration of oestradiol induces atresia of the cyst by suppressing LH and FSH support via a steroid negative feedback mechanism. In a study by Douthwaite and Dobson (2000), 22 cows with follicular cysts and 14 cows with luteal cysts were treated with a progesterone intra-vaginal device[2]. The conception rates after first insemination after treatment were 18% and 28%, respectively.

Other combinations used include hCG and progesterone (Alanko and Katila, 1980; Kupfer *et al*., 1991), simultaneous treatment with GnRH and prostaglandins (Dinsmore *et al*., 1990), GnRH and prostaglandins 7 days later (Fricke and Wiltbank, 1999), and double injections of GnRH at a 7-day interval (Eissa and El-Belely, 1995).

2.3. Concluding remarks

Most studies of the treatment of COD have primarily focused on the restoration of regular ovarian cyclicity, ultimately resulting in pregnancy. It is clear that the results of treatment differ considerably between studies, which may be due to a herd effect. How a farmer manages his/her farm influences, for instance, the severity and duration of NEB, and might be a major factor contributing to the herd effect.

A few studies investigated the influence of when the diagnosis was made on treatment response. Steinbauer (1985) recommended treatment only after day 50 *post partum*, because of possible spontaneous regression of the cyst. Dinsmore *et al*. (1989) did not find major differences between clinical response to treatments given before or after 35 days *post partum.*

To avoid unnecessary economic losses, it is clear that cows have to re-establish ovarian cyclity as soon as possible after treatment (Scholl *et al*., 1992). The post-treatment insemination success rate is also important. Thus, besides the costs of treatment, the intervals between treatment and insemination and between insemination and conception, which reflect reproductive performance after treatment, may contribute to economic losses due to COD.

3. Genetics

A number of studies have investigated the heritability of fertility and production traits (see Table 1). The incidence and the heritability of COD ranges between 1.6% and 9.1% and 0 and 13%, respectively, but these figures are generally based on small data sets. The variance in these results may be due to differences in study design, particularly data collection, size of data sets, statistical evaluation, and breeds. A standardized way to

[2] PRID®, Sanofi

diagnose and register COD is essential, which is not possible in analyses of nationally recorded computerized data sets of veterinary treatments. However, meaningful statistical analyses need large data sets, because often many sires are involved, with a limited number of daughters per sire. Moreover, because of possible correlations with milk production traits, it is likely that differences between breeds exist. The relationship between COD and production traits has hardly been studied and results are conflicting (Table 1).

TABLE 1. Summary of studies of genetic parameters of COD and relationship between COD and milk production traits. Par. = parity.

author	**study period**	**number of lactations**	**incidence (%)**	**heritability**	**correlation of COD with milk production traits**
Kirk *et al.*, 1982	1974-80	2112	6.15	influence of two bulls	
Solbu, 1984	1979-81	variable	1.6	0	fat%/max. daily milk yield positive
Cole *et al.*, 1986	1963-83	902 (par.1)	0→44	pedigree analysis (n=30)	
		1344 (> par.1)	0→52	recessive inheritance	
Lin *et al.*, 1989	1981-83	7712	par.1-2-3 resp.	par.1-2-3 resp.	
			4.2 - 9.3 - 11.6	0.12 - 0.08 - 0.02	
Ashmawy *et al.*,	1977-87	249 (par.1)	34	0.15	
1990		152 (par.2)	42	0.11	
Lyons and Freeman, 1991	??	11008	5.8	0.1	-0.01 (yield), 0.24 (fat)
Distl, 1992	1986-88	5080	5.4 (Braunvieh)	0.03	
			8.9 (Fleckvieh)		
Mäntysaari *et al.*, 1993	1983-85	28277	6	0.03	
Uribe *et al.*, 1995	1989-91	7416	7.4 (par.1)	0.13 (par.1)	small negative with yield,
			9.1 (all par.)	0.08 (all par.)	inconsistent with fat, protein
van Dorp *et al.*, 1998	1994-95	4368	3.1	0.02	0.23 yield (par.1)

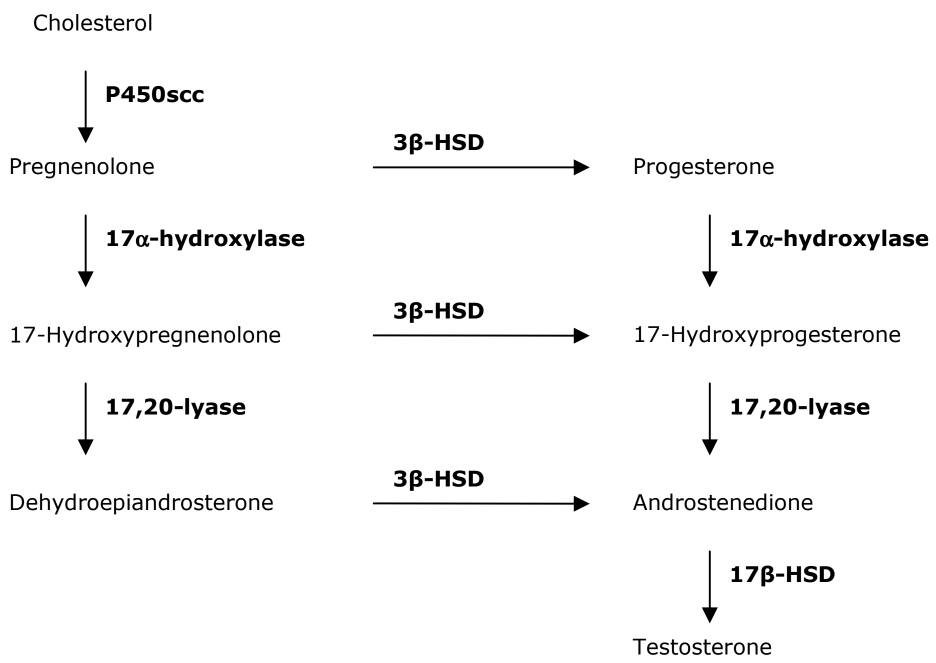
4. Polycystic ovarian syndrome

Polycystic ovarian syndrome (PCOS) is a heterogeneous endocrine disorder which is considered to be the most common cause of anovulatory infertility in women. It is characterized by hyperandrogenism and chronic anovulation (Franks *et al.*, 1997;

Urbanek *et al*., 1999). PCOS shows differences from, but also similarities to, COD in cows. For this reason, it is of interest to describe the metabolic, endocrinological, and genetic disorders in women with PCOS, to see whether analogous mechanisms are involved.

PCOS is a leading cause of female infertility and is associated with polycystic ovaries, hirsutism, obesity, and insulin resistance (Urbanek *et al*., 1999). The ultrasound finding of polycystic ovaries, defined as presence of eight or more subcapsular follicular cysts ≤ 10 mm in diameter and increased ovarian stroma, is not a prerequisite for the diagnosis of PCOS. This because less than 30% of an unsolicited population may have polycystic ovaries on ultrasound examination, and many of these women have normal androgen levels and regular menstrual cycles (Polson *et al*., 1988). Approximately 5% of women of reproductive age have evidence of clinical androgen excess (based on hirsutism or acne) or biochemical androgen excess (based on circulating androgen levels) in conjunction with chronic anovulation (Knochenhauer *et al.,* 1998; Diamanti-Kandarakis *et al*., 1999; Solomon, 1999).

FIGURE 3. Part of the steroid biosynthetic pathway in adrenal cortex and theca cells of ovaries. P450scc = cholesterol side chain cleavage; 3β-HSD = 3β-hydroxysteroid dehydrogenase and Δ^5 –isomerase; 17β-HSD = 17β-hydroxysteroid dehydrogenase; 17α-hydroxylase activity and 17,20 lyase activity is mediated by P450scc17α (Xita *et al*., 2002).



Hyperandrogenism usually prevails in patients with PCOS (androstenedione and/or testosterone increased), but a predominance of LH in the LH/FSH ratio is also seen. Moreover, decreased levels of sex hormone-binding globulin (SHBG) result in increased levels of free testosterone, 17α-hydroxy progesterone (17-OHP), androstenedione, and dehydroepiandrosterone (DHEAS). A scheme of part of the steroid biosynthetic pathway is shown in Figure 3.

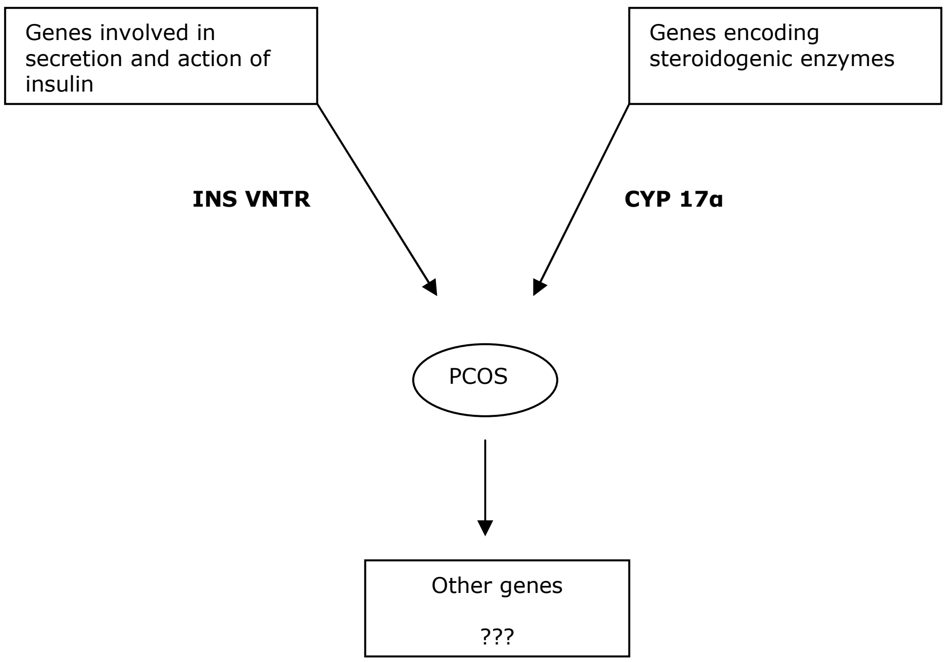
Polycystic ovarian syndrome is accompanied by metabolic derangements such as increased insulin concentrations, hyperinsulinaemia and insulin resistance, and hyperlipidaemia resulting in hypertension, diabetes, or cardiovascular disease (Acién *et al*., 1999). The combination of these disorders and their relative expression are highly variable between individuals, including first-degree relatives (Abbott *et al*., 2002). Thus, PCOS is a complex disorder involving endocrinological and metabolic disturbances.

The pathogenesis of PCOS in women and COD in cows has not yet been elucidated, despite numerous studies of the metabolic and endocrinological disturbances in patients with PCOS and of the relationship between metabolic changes in early lactation and impaired reproductive performance in dairy cows (Jorritsma *et al*., 2002).

4.1. Genetic basis of PCOS

The genetic basis of PCOS remains unknown, but many studies have investigated possible candidate genes, such as the genes involved in steroid hormone synthesis and action (*CYP11α, CYP17*, androgen receptor and SHBG), gonadotrophin action and regulation (follistatin), and carbohydrate metabolism (insulin, insulin receptor, insulin receptor substrate proteins) (Legro and Strauss *et al*., 2002) (Figure 4). So far, there is evidence that a minisatellite of the insulin gene, *INS VNTR* (insulin variable number of tandem repeats), and *CYP11α*, which encodes the enzyme cytochrome P450scc (cholesterol side chain cleavage), which catalyses the initial rate-limiting step at the start of the steroid biosynthetic pathway, namely the conversion of cholesterol to pregnenolone, are associated with PCOS (Figure 3). However, because several pathways are implicated in the aetiology of PCOS, it will be difficult to identify the contributing genes (Urbanek *et al*., 1999).

FIGURE 4. Suggested pathogenic mechanisms in the development of PCOS (Xita *et al*., 2002). INS VNTR= insulin variable number of tandem repeats; CYP17α=cytochrome P450 17α-hydroxylase/17,20-lyase (Xita *et al*., 2002).



4.2. PCOS and COD

There are several phenotype differences between PCOS and COD. A main one might be the fact that cows, which do not recover from COD within a few months, are culled. Therefore, in veterinary practice little is known about protracted COD and its endocrine and metabolic consequences. Moreover, the endocrinological and metabolic disorders in women with PCOS vary considerably and give rise to several phenotypes. In contrast to COD, anovulation and hyperandrogenism always occur in PCOS.

The metabolic disorders seen in women with PCOS are not very prominent in cows with COD. Opsomer *et al*. (1999a) concluded that because cows with COD had a significantly lower insulin response to a standard glucose load, insulin could be a factor in the pathogenesis of COD in dairy cows. But neither insulin resistance, nor elevated testosterone levels, as observed in PCOS, were present in cows with COD.

PCOS and COD are similar in terms of the presence of cystic structures in the ovaries, which are always associated with anovulation in women and often in dairy cows.

Moreover, as in PCOS, metabolic hormones such as insulin, IGFs, GH, or leptin (Goumenou *et al*., 2003), may play a role in the pathogenesis of COD because of their direct or indirect effects on GnRH in the hypothalamus. Thus, COD could possibly serve as an animal model of PCOS.

5. Scope of the thesis

5.1. Characteristics of field studies

Cystic ovarian disease has been studied extensively in an attempt to unravel the patho-physiological process leading to the condition and to identify the host and environmental factors affecting that process. The characteristics of field trials and well-organized clinical trials are briefly described, with emphasis on the problems encountered when studying COD.

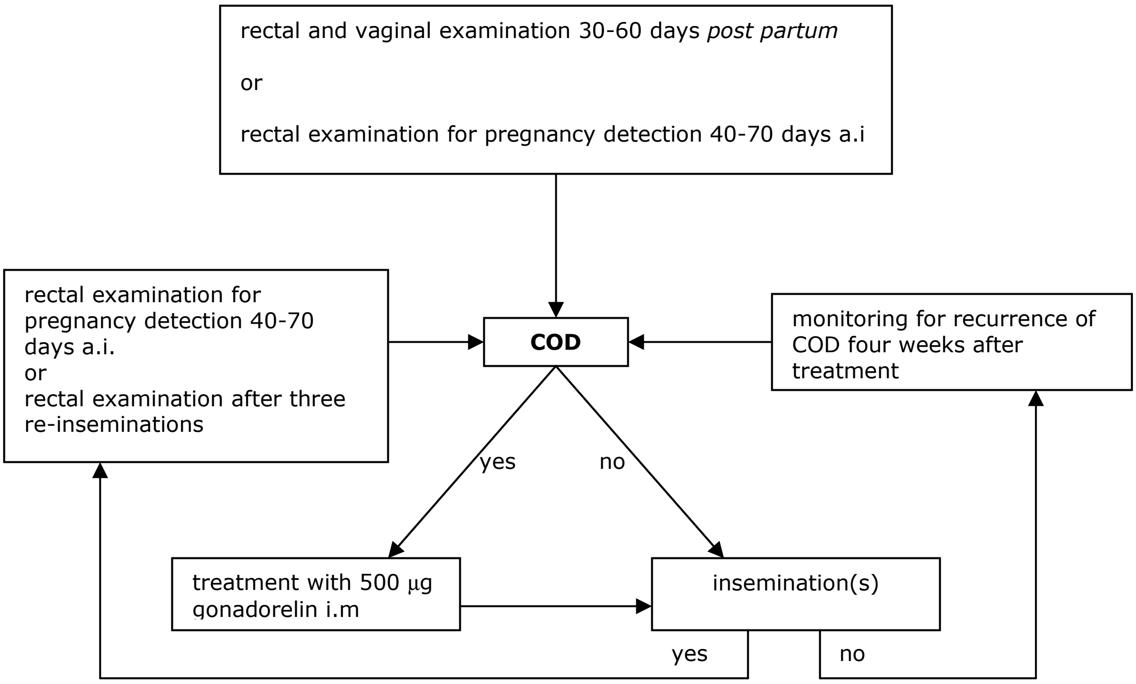
A field trial involves subjects "in the field", that is, under husbandry and management practices typical of those under which the procedure is intended to be used (operational conditions) (Thrusfield, 1995). For instance, field trials can be used to investigate the effectiveness of treatments, or to monitor the incidence of certain diseases. An advantage of a field trial, compared to a clinical trial, is that it is easier to set up large groups on many farms. However, the statistical analysis may be more difficult than in a controlled in-station clinical trial, because complex models have to be used in order to adjust for potential bias. A controlled in-station clinical trial requires a control group, with group allocation made according to a blinded procedure (Thrusfield, 1995). In practice, the feasibility of the study design often determines the sort of trial.

Survival analysis is often used in field and clinical trials, because animals disappear from or enter the study population during the study period (animals at risk), and because the time until the event occurs (survival time) is important (Frankena and Graat, 1997). The fact that it is impossible to reliably predict if and when a cow will develop COD makes controlled research very difficult and therefore the results described in this thesis are based on field data, with survival analysis being used as an important analytical tool.

5.2. Fertility protocol

The field data were collected over a period of 10 years (1987-1996) on farms served by one veterinary practice in the Netherlands. The use of a standardized fertility examination protocol in combination with an extensive and uniform recording of gynaecological findings has created a unique data set.

FIGURE 5. Flow chart of the fertility examination protocol used in four-weekly visits. COD was characterized as the presence of one or more ovarian follicular structures with a diameter of at least 2.5 cm in diameter detected on one or both ovaries in the absence of a *corpus luteum* (a.i.=after insemination). Cows without COD that were not inseminated were culled (not included in the scheme).



The fertility examination protocol is summarized in Figure 5. This protocol is highly practical and allows a uniform way of working by all practitioners involved. Moreover, almost all cows are investigated *post partum* with registration of all data concerning calving, oestrus, and insemination, including gynaecological findings and treatments, in the VAMPP computer program (Noordhuizen and Buurman, 1984). The first gynaecological examination is usually performed between about 30 and 60 days *post partum.* With this fertility examination protocol, COD can be diagnosed after one or more examinations before the first insemination, after examination for pregnancy detection, or after examination for repeat breeding. Further, if not inseminated, COD-positive cows are re-examined for recurrence of COD 4 weeks later.

5.3. Subjects of study

In general, the effects of treatment have not been assessed in a quantitative way, but more in terms of veterinarian opinion. No large-scale studies have been done in the Netherlands of the results of treatment of COD with GnRH, particularly with regard to reproductive performance after treatment. The field data described in this thesis may contribute to scientific but also more practical knowledge about aspects of the treatment of COD in cows kept under certain herd management conditions. Not only the results of treatment of COD with GnRH, but also the prospects of treated cows are relevant. Results are described in Chapter 2 and 3.

Studies of the heritability of COD in dairy cows have not been carried out in the Netherlands. The heritability of COD, but also the genetic linkage between COD and milk production traits, have strong implications for genetic selection for COD, particularly because in the study period (1987 – 1996) the 305-day milk yield increased from 6300 kg (milk fat 4.35%, milk protein 3.40%) to 7705 kg (milk fat 4.41%, milk protein 3.47%) (National Breeding Organization, 2002), and because a high milk production may be associated with COD. The size and quality of the data set made it possible to eliminate many of earlier mentioned problems with regard to the study design (data collection, size of data sets, statistical evaluation). Results on this aspect can be found in Chapter 4.

NEB is a hot topic in herd management (Brand and Varner, 1997), particularly in relation to reproductive performance. The relationship between COD and NEB was investigated, especially with regard to incidence, treatment, and fertility parameters. In this study we restricted ourselves to parameters measurable from recorded milk production data, particularly the fat-to-protein ratio (as indicated by Dobbelaar *et al*., 1998) and the daily change in the milk fat and protein content in early lactation (see de Vries and Veerkamp, 2000). The aim of this study was to investigate whether proxy parameters of NEB, based on data obtained from the first and second milk recordings after calving, are significantly associated with the incidence of clinical COD and reproductive performance after hormonal treatment with GnRH (Chapter 5 and 6).

In conclusion, the studies described in this thesis investigated aspects of treatment with GnRH, the heritability of COD in dairy cows, the genetic linkage between COD and milk production traits, and epidemiological aspects such as possible relationships between the incidence of COD and NEB, and between reproductive performance after treatment of COD and NEB. In the general discussion (chapter 7) findings are discussed, with emphasis on their practical implications.

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Chapter 2

Treatment of cystic ovarian disease in dairy cows with gonadotrophin-releasing hormone: a field study

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Abstract

In a field trial in the Netherlands 765 lactating dairy cows were treated in 869 lactations with 500 µg gonadotrophin-releasing hormone (gonadorelin[3] i.m.) for cystic ovarian disease (COD) between June 1987 and April 1996. COD was defined as the presence of a large follicle (> 2.5 cm) in one or both of the ovaries in the absence of a *corpus luteum*. Two treatment groups were formed, based on the interval from parturition to first treatment, with day 60 being the cut-off between groups 1 and 2. The aim of this study is to examine whether there is a relationship between the moment of diagnosis and treatment (before or after day 60 *post partum*) and its result. In group 1 90.0% of first treatments were effective and in group 2 93.3% (p=0.08). This was reflected by the slightly higher number of treatments needed for animals in group 1 (1.11 versus 1.07, p=0.08). The efficacy rate after one treatment did not significantly differ (1.63 versus 1.69, p=0.40), nor did the interval between 1st insemination after treatment and conception (p<0.63).

In conclusion, it can be stated that gonadorelin is effective as therapy irrespective of the timing of diagnosis and treatment.

Key words: gonadorelin, cystic, ovary, recovery, cow.

[3] Janssen - Cilag (originally Akzo - Intervet)

Chapter 3

Fertility parameters of dairy cows with cystic ovarian disease after treatment with gonadotrophin-releasing hormone

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Abstract

A field trial was carried out in the Netherlands to evaluate the effect of treating dairy cows with 500 μg gonadorelin (Fertagyl; Janssen-Cilag) for cystic ovarian disease (COD) during 925 lactations. Fertility parameters of this group were compared with those of a control group of 13,869 lactations without COD. In this study, COD was defined as the persistence of an anovulatory follicular structure with a diameter of more than 2.5 cm in the absence of a *corpus luteum*, resulting in aberrant reproductive functioning. The intervals between parturition and 1st insemination and between parturition and conception were significantly shorter ($P<0.001$) in the normal cows, but the interval between 1st insemination and conception was not ($P=0.31$). The overall conception rate tended to be higher ($P<0.10$) in the normal cows, but the conception rate after 1st insemination was significantly higher ($P<0.001$) and the number of services per conception was significantly lower ($P=0.008$).

In conclusion, it can be stated that a prolonged interval between parturition and 1st insemination is responsible for the delayed interval between parturition and conception. Regular fertility monitoring can minimize the number of days open of cows with COD because treatment with GnRH does not result in a prolonged insemination-conception interval.

Key words: gonadorelin, cod, cystic, ovary, cow, treatment, fertility

Chapter 4

Genetic parameters for cystic ovarian disease in Dutch black and white dairy cattle

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Abstract

Cystic ovarian disease (COD) is one of the most frequently diagnosed gynaecological findings in dairy cattle. It causes temporary infertility and is likely to affect reproduction as well as production parameters in affected cows. The objectives of this study were to investigate the heritability of COD in a Dutch Black and White population and to estimate the genetic and phenotypic relation with milk production traits. In the data set used, the overall incidence of COD was 7.7% (1204 COD diagnoses in 15,562 lactations). The farm incidence varied between 1.9 and 11.3%. The estimated heritabilities on the underlying and observable scale were 0.102 and 0.087, respectively. The genetic correlations between COD and 305-day milk, fat and protein yields were 0.35, 0.38 and 0.44, respectively. We concluded that a genetic predisposition for COD exists in Dutch Black and White dairy cattle. The genetic correlations between COD and yield traits indicate that ongoing selection for production will increase the incidence of COD.

Key words**:** cystic ovarian disease, dairy cattle, genetic parameters

Abbreviation key: COD = cystic ovarian disease; VAMPP = Veterinary Automated Management and Production Program.

Chapter 5

Milk production parameters in early lactation: potential risk factors of cystic ovarian disease in Dutch dairy cows

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Livest. Prod. Sci. (2003) 81:25-33

Abstract

The aim of this field study was to investigate whether the incidence of cystic ovarian disease (COD) in dairy cows was related to milk production parameters (milk yield, milk fat and protein) in early lactation with special emphasis on the negative energy balance (NEB). The diagnosis of COD was made by rectal palpation performed by veterinarians experienced in gynaecology. Data used for this field study were collected from November 1986 onwards. The data set consisted of 6,911 lactations (3,891 cows) on 29 herds including 600 cases of COD (8.7%). Lactations in the COD group had higher average maximum productions of milk, energy corrected milk, milk fat and protein than lactations in the non-COD group. The average maximum milk fat:protein ratio did not significantly differ between both groups. Daily change of the average maximum milk fat and protein percentage and of total milk fat and protein yield were calculated and divided into four groups depending on the day of first milk recording. No significant relations were found between changes in milk fat and protein percentage and total milk fat and protein yield, and the incidence of COD. It is concluded that elevated average peak milk yield, milk fat and protein yield in early lactation are risk factors for the incidence of COD, but the average milk fat:protein ratio is not. In this field study changes in milk fat percentage and milk fat yield, indicative for the presence of NEB, did not show a relation with the incidence of COD in early lactating cows.

Key words: Dairy cow, cystic ovarian disease, COD, production, negative energy balance, NEB, risk factors.

Chapter 6

Influence of negative energy balance on the reproductive performance aftert treatment of cystic ovarian disease with gonadotrophin-releasing hormone in dairy cows

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Submitted

Abstract

The aim of this study was to investigate whether a significant relation exists between the presence of NEB in cows early in lactation and the reproductive performance after treatment with GnRH of cows with clinical COD. Reproductive performance after treatment was assessed from the interval between treatment and first insemination (ITFI) and the interval between treatment and conception (ITC). Based on the outcome of the daily change of milk fat yield (∆fatg) between the first and second milk recordings *post partum*, cows were considered to have passed the NEB nadir (positive ∆fatg) or not (negative ∆fatg). Lactations (n = 430) were divided into 4 groups according to the interval between calving and first milk recording (ICMR). The effect of ∆fatg was significant for group 1 and group 3 (ICMR on 0 to 9 days and 20 to 29 days p.p., respectively) with an increased and a decreased probability of being inseminated the first day after treatment (ITFI), respectively. However, no significant interaction was found between ∆fatg and the groups with regard to ITC. The net result was that cows with clinical COD and treated with GnRH that had a NEB nadir early in lactation had a 3-7 day shorter day-open interval than cows with NEB nadir 10 to 20 days later.

Key words: Dairy cattle; Cystic ovarian disease; COD; Treatment; GnRH; Reproductive performance; NEB;

Chapter 7

General discussion

G.A. Hooijer

General discussion

In this thesis, three main aspects of cystic ovarian disease (COD) in dairy cattle were studied:

- the results of treatment with gonadorelin[4], and reproductive performance after treatment;
- the heritability of COD together with estimation of the genetic and phenotypic relationships with milk production traits;
- the role of a negative energy balance (NEB) *post partum* regarding the incidence of clinical COD and reproductive performance after treatment.

All studies were based on the same standard fertility examination protocol (chapter 1) in combination with uniform recording of gynaecological findings. Large-scale studies, based on data of centrally recorded health programs, involve many practitioners, which makes it difficult to guarantee that a uniform approach is used. In our longitudinal field studies, the diagnosis of COD was made by rectal palpation performed by veterinarians with experience in gynaecology. The diagnosis was made in accordance with the study design used in other epidemiological studies (Laporte *et al*., 1994; Eicker *et al*., 1995; van Dorp *et al.*, 1998; van Dorp *et al*., 1999), and with current veterinary practitice. We did not use ultrasonography on a large scale during the study period because this technique was not yet available at the start of our studies, and we were not interested in trying to distinguish between follicular and luteal cysts because both types of cysts are equally responsive to GnRH (Dinsmore *et al*., 1989; Day, 1991).

Generally, cows were not investigated before 30 days *post partum* because in our veterinary practice cows are, except in severe cases, not treated for endometritis, or COD in the first 4 weeks of lactation. Improving herd management in the peri-parturient period (feeding, oestrus detection, oestrus recording) is the best way that farmers can ensure that lactation starts normally, ultimately resulting in a timely resumption of ovarian cyclicity. However, if herd management appears to be inadequate, it may be useful to examine cows earlier in order to gain insight into *corpus luteum* activity, which is determined in part by the postpartum nadir in NEB (Canfield and Butler, 1990).

Even though the fertility examination protocol was used, 25% of the diagnoses for cows with a first diagnosis COD after day 60 *post partum* (group 2, chapter 2) were made after 107 days *post partum* (Q3 = 107). These cows were first examined 4 weeks earlier without COD being diagnosed. In addition, a number of these cows were inseminated before the diagnosis COD was established, resulting in a lower conception

[4] Fertagyl® Janssen-Cilag (originally Akzo-Intervet)

rate and more services per conception. Without regular fertility monitoring, more inseminations are likely to be ineffective, resulting in more days open.

Because all the studies of this thesis were based on the same protocol, it is essential to understand the setting of this protocol. While we do not presume that there are no other approaches for monitoring cows *post partum*, we did not find any in the literature. Our examination protocol has proven very helpful in the regular monitoring of herd management. We were able to analyse several aspects of COD that may be relevant not only for veterinary practitioners but also for scientists. The treatment of COD with GnRH is discussed (section 1) not only with regard to parameters of reproductive performance after treatment, but also in relation to NEB in cows *post partum* and reproductive performance after treatment.

In another study we proved a genetic predisposition for COD in cows. Moreover, the incidence of COD appears to be correlated with milk production traits. The implications of these results for cattle-breeding programmes and suggestions for further research are briefly discussed in section 2.

In section 3, the proxy parameters used in our studies are discussed extensively, because conclusions have been made about the relationship between NEB and reproductive performance after GnRH treatment and the incidence of COD, based on these proxy parameters for NEB.

1. Treatment

Three studies of the treatment of clinical COD with GnRH were carried out, with as aim

- to evaluate the results of treatment of two groups of cows regarding the day of treatment *post partum*. The first group consisted of cows in which the diagnosis of COD was made earlier than 60 days *post partum* and the second group included cows in which the diagnosis was made after day 60 (chapter 2);
- to compare reproductive performance of treated cows with that of non-affected cows (chapter 3);
- to investigate possible relationships between reproductive performance after treatment and NEB (chapter 6); the results of this study are briefly discussed in section 3.

Generally, treatment results have to be judged in relation to criteria for successful treatment. Some studies defined a palpable *corpus luteum* and/or the absence of a cyst (Dinsmore *et al*, 1989; Jou *et al.*, 1999) or recovery of a normal oestrous cycle (Day, 1991) as a positive response. However, at least two cycles should be evaluated in order to judge whether or not a cow has recovered regular cyclicity. In clinical trials, in particular, the determination of plasma progesterone levels, whether or not in combination with ultrasonography, is helpful in deducing morphological changes of the ovaries. In our field study, described in chapter 2, treatment of clinical COD was considered to be effective if at least one of the following criteria were met:

- a *corpus luteum* and/or follicle < 2.5 cm was found on one or both ovaries;
- disappearance of the cyst without further palpable structures on the ovaries;
- calving as a result of insemination after treatment, in those cases lacking further information;
- no diagnosis of recurrence during the same lactation ("palpable" corpus luteum does not necessarily mean that endocrine regulation is restored).

The main conclusion of this study was that GnRH is effective as treatment for clinical COD in dairy cows regardless of when treatment is started after day 30 *post partum*. About 90% of the cows with clinical COD became pregnant 0 to 356 days after treatment with GnRH. We found a tendency for better recovery after first treatment in group 2 (treatment ≥ 60 days *post partum*, P=0.08) than in group 1 (treatment between 30 and 60 days *post partum*). However, the efficacy (= pregnancy) rate after 1^{st} treatment (P=0.4) did not differ between the groups. This might be explained by the findings of a study of Eissa and El-Belely (1995). In that study, the reproductive performance of cows with COD after a single treatment was compared with that after a double treatment, in which the second treatment took place 7 days after the first treatment (Table 1). The skim milk progesterone concentration after a single treatment did not exceed 3 ng/ml, whereas it increased to over 5 ng/ml 4-6 days after the second injection. This points to there being complete luteinization of the cyst, with a decrease in progesterone level at the end of the sampling period representing regression of luteal tissues. The milk progesterone concentration after treatment of COD was positively correlated with the pregnancy rate after treatment of COD. Thus, progesterone concentrations after treatment are positively correlated with parameters of reproductive performance. The obeservation that in our study the faster resumption of ovarian cyclicity in group 2 than in group 1 did not result in a higher pregnancy rate in cows of group 2 may be because luteinization was insufficient resulting in lowered progesterone levels. Moreover, energy balance is positively associated with the plasma level of metabolic hormones, such as IGF-I, which is positively correlated with the number of LH receptors in the theca cells and with LH-induced production of progesterone in vitro (Stewart *et al.*, 1995). Thus, a NEB may play a role in reproductive performance after treatment of cows with COD with GnRH. The relationship between treatment results and NEB is discussed in section 3.

TABLE 1. Comparative reproductive performances of cystic cows treated with a GnRH analogue* (single or double injections 7 days later) or in untreated controls (Eissa and El-Belely, 1995).

treatment	n	no. of cows responded	interval calving to 1st ins. (days)	interval treatment to conception (days)	interval calving to conception (days)	no.of cows conceived on 1st ins.
single inj.	8	5 (62.5%)[a]	103.4 ± 9.5[a]	75.6 ± 4.2[a]	104.3 ± 9.2[a]	3 (37.5%)[a]
double inj.	11	9 (81.8%)[b]	69.1 ± 3.2[c]	28.3 ± 1.8[c]	82.6 ± 5.3[b]	7 (3.6%)[b]
single + double inj.	19	14 (73.7%)[a]	86.5 ± 5.2[b]	43.2 ± 3.3[c]	93.5 ± 6.4[c]	10 (52.6%)[b]
control	7	3 (42.9%)[c]	108.6 ± 8.6[a]	72.5 ± 6.3[a]	131.7 ± 11.3[a]	3 (42.9%)[a]

* Receptal® (Akzo-Intervet); a & b: significant with P<0.05; a & c: significant with P<0.1.

We can conclude that, in studies of COD, it is relevant to distinguish between recovery from clinical COD, according to valid criteria, and the pregnancy rate after treatment, reflected by parameters such as the interval between treatment and conception. This is illustrated by the fact that no field studies have included one of our criteria of recovery, namely the criterion of no recurrence during the same lactation.

In another study (chapter 3) the interval between calving and 1st insemination and the interval between calving and conception in dairy cows were negatively influenced by COD compared with those in an unaffected control group (n=925 and n=13,869, respectively; P=0.001 and 0.008, respectively). However, the main conclusion is that the insemination-to-conception interval is not prolonged in cows treated for clinical COD with GnRH. The prolonged interval between calving and 1st insemination in the COD group (median 89 versus 77 days) was the most important factor that resulted in a delayed conception. Thus, the early diagnosis and treatment of COD diminishes the number of days open and the number of services per conception and therefore reduces the economic impact of COD.

Once a cyst is diagnosed, the decision to treat it, or not, has to be made. White and Erb (1980) concluded on the basis of a decision analysis that, using a cost of $2 per day not cycling over 70 days, treatment with GnRH on day 8 *post partum* was cheaper than no treatment. Scholl *et al*. (1992) reported that it was economically favourable to treat COD with GnRH between day 60 and day 116 *post partum.* Lower replacement heifer purchase costs would favour the no-treatment decision. Steinbauer (1985) recommended treatment after 50 days *post partum*, because only repeated examinations allow clarification of persistence or spontaneous regression of cysts. Dinsmore *et al*. (1989) and Seguin *et al*. (1976) concluded that the clinical response after treatment, defined by the absence of a cyst or an increase in serum progesterone concentration, before 30 days *post partum* did not differ from that of cows treated later. In a study of Jou *et al*. (1999), no relation was found between *corpus luteum* detection or cyst disappearance in a group cows with COD treated with saline (n=22) compared with a group treated with GnRH (n=17), indicating spontaneous recovery on a large scale. However, in that study a high level of censored data was present. In our study, we corrected calculations of the conception rate, conception rate after 1st insemination, and services per conception for the interval between calving and 1st insemination (multivariate analysis). After this correction, the conception rate of the COD group was no longer statistically different from that of normal cows, but the number of services per conception was higher because all inseminations before COD was diagnosed were included. Thus, spontaneous full recovery on a large scale, as suggested by Jou *et al*. (1999), seems unlikely when taking our results on conception rate into account.

Clinical COD can be treated with GnRH without using ultrasonography as a diagnostic tool. Extensive recording of all gynaecological findings during regular visits, including evaluation of treatments, is essential for monitoring the reproductive performance of dairy herds and will result in better preventive health care. In this context, in order to understand the results of other studies on the treatment of COD, it should be borne in mind that disease events may be recorded more accurately if the herds are involved in a particular study and are regularly visited than in a continuous national health recording program such as described by Mäntsysaari *et al*. (1993).

In practice, it is unusual to evaluate treatments on a large scale, based on reliable data, yet. From both a scientific and practical point of view it is essential to collect data in order to assess current treatments for COD. However, this requires agreement on uniform diagnostic criteria, treatments, and criteria of successful treatment.

2. Heritability

Current dairy cattle breeding programmes are largely focused on milk production. In recent years the relatively high culling percentage (between 30% and 40%) in Dutch dairy herds has increasingly become a subject of discussion. Nowadays, there is a tendency to stimulate conversion from an intensive to a more extensive Dutch livestock industry, which may increase the lifespan of dairy cows. In our study, 9.1% of all cows with COD were infertile and subsequently culled (table 1, chapter 3), and the incidence of COD in the second and third lactations was 2.5% higher than in heifers (table 2, chapter 4). In order to change policy regarding culling due to COD, knowledge is needed of the heritability of COD and possible genetic correlations between milk production traits and COD. In our study of genetic variance (chapter 4), the trait COD was considered as the binary expression (present/absent) of an underlying (unobservable) variable that is influenced by genetic and environmental factors. The threshold model, used to estimate variance in binary traits, assumes that phenotypic expression of a binary trait is determined by an underlying Normally distributed trait, called the liability. If the underlying trait, for example, is related to endocrine functioning, then higher than threshold values of the underlying trait would result in an affected animal. The value of the liability, which is assumed to differentiate between animals with phenotypes 0 and 1, is related to the incidence. In our study, the incidence of COD increased from 3.0% in 1987 to 9.5% in 1996. The farm incidence, however, varied between 1.9 and 11.3%. The computed heritability (h^2) of COD on the underlying scale was 0.10 and, after transformation to a value on the observable scale (Cameron, 1997), 0.09. The variation in the farm incidence, but also the value of the h^2, illustrates clearly the existence of environmental effects (including "farmer" effects) in the pathogenesis of COD.

In our study, correlations existed between milk production traits and the incidence of clinical COD. Other studies showed variable results concerning the relationship between milk yield and COD (chapter 1, table 1). Currently, livestock breeding programmes are affected by both political and economic issues. From an economic point of view, the promising results obtained with GnRH as treatment for COD indicate that there is no need to exclude COD cows from breeding programmes. This will probably result in an increased incidence of clinical COD in the near future, not only because of the possibility of repeated COD in successive lactations, but also because of the heritability of COD and its correlation with milk production. Increased milk production requires better herd management because the altered metabolic demands of peri-parturient cows may favour the development of COD. However, COD might be the cause of an increased milk production (Kesler and Garverick, 1982), possibly by a linked inheritance. Thus, the cause-effect relation of milk production traits and COD might exist at a metabolic and a genetic level.

We demonstrated a genetic predisposition for COD in Dutch dairy cattle together with a substantial correlation between COD and milk production traits. The main goal has to be to find candidate genes for COD, which might make marker-assisted selection in breeding programmes possible. Moreover, a linked inheritance of COD and milk production traits would confirm at a molecular level the correlations found between milk production and COD in the field. The resemblance between PCOS in humans and COD in cattle warrants the exchange of research findings on these two diseases.

3. Negative Energy Balance

Several studies have investigated the relation between NEB and reproductive performance after calving. Reproductive performance is a collective term involving, among other things, resumption of ovarian cyclicity after calving, uterine involution, oestrus expression, conception rates, and disturbances such as COD and endometritis. A diminished reproductive performance is of economic interest because of a higher culling risk, treatment costs, and more days open. However, few studies have focused on the relationship between NEB and COD. In this thesis, the incidence of clinical COD (chapter 5) and the reproductive performance after treatment with GnRH in relation to NEB (chapter 6) were analysed.

In field studies it is impossible to measure accurately on a large scale the energy balance of the peri-parturient or *post partum* cow. For instance, it is difficult to collect an adequate number of samples in order to detect NEB. An incidence of clinical COD of 0.10 means that on average 10 animals have to be investigated in order to detect one case of COD. Experimental research with respiration chambers to measure energy balance is impractical, partly because of the low incidence of COD. Although ACTH can be used to induce COD (Ribadu *et al*., 2000), reliable induction models are not available.

For these reasons, proxy parameters of NEB, determined from the milk production data of the first and second milk recordings *post partum,* were used. However, there is no consensus about the sensitivity and specificity of proxy parameters for NEB based on milk recording data. In one study, a cut-off value of > 1.5 for the fat-to-protein ratio in milk collected during the first milk recording after calving proved to be a better indicator of NEB than the Ketolac® milk test[5], the sodium nitroprusside test[6] (acetoacetate) or plasma beta-hydroxybutyric acid level (Heuer *et al*., 2000). De Vries and Veerkamp (2000) calculated changes in milk yield, percentage of milk fat and protein, milk fat and protein yield, and milk fat-to-protein ratio for 470 first parity heifers at 2, 6, 8, 11, and 15 weeks *post partum.* They concluded that a decrease in milk fat percentage during

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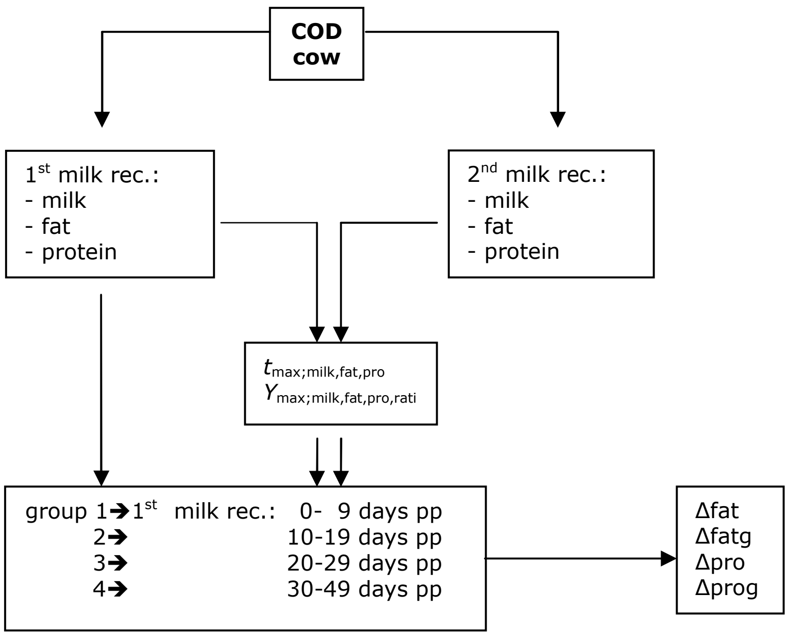
[5] Hoechst, Unterschleissheim, Germany

[6] Utrecht powder (Geishauser *et al*., 1998)

early lactation might serve as an indicator of energy balance. Based on that study, we calculated the daily change in milk fat and protein yield and milk fat and protein percentage between the first and second milk recordings *post partum*. However, in field studies, there is considerable variation not only in when these recordings are made, but also in the curve of fat and protein content of the milk of individual cows. This is clearly shown in a study of Wilmink (1987), in which age at calving of heifers influenced the production of milk, milk fat, and protein. Moreover, second and later parity cows had higher milk yields than heifers, with the greatest difference occurring at the beginning of lactation.

FIGURE 1. Analysis of milk production parameters of the first and second milk recordings *post partum* of cows with cystic ovarian disease (COD). t_{max} = day *post partum* in which the predicted peak milk yield is reached; Y_{max} = peak milk yield in kg/day (milk) or gr/day (fat & protein); Δfat, Δpro = daily change in milk fat and protein percentage, respectively; Δfatg, Δprog = daily change in gram milk fat and protein, respectively.



It is clear that it is not relevant to analyse the daily changes in milk fat and protein yield and milk fat and protein percentages between two milk recordings without correcting for when in lactation these milk recordings were made. We dealt with this problem (chapter 5, 6) by calculating Y_{max} and t_{max}. Based on the data of the first and second milk recordings *post partum*, for each cow the peak milk yield (Y_{max}) after calving and the day on which the predicted peak milk yield (t_{max}) was reached (Koops, 2001), were calculated.

Cows were initially divided into five classes depending on when the first milk recording occurred, resulting in four groups (Figure 1). The daily changes in milk fat and protein yield and in milk fat and protein percentage were analysed in relation to these groups. The daily change in milk fat content was used to determine whether cows had passed the NEB nadir (positive daily change) or not (negative daily change) between the first and second milk recordings. The purpose of this analysis was to gain more precise insight into the production of the cow during early lactation compared with that obtained by analysing the first test day milk only.

In our study, neither the average maximum milk fat:protein ratio nor the average daily change in milk fat and protein percentage or milk fat and protein yield, was related to the incidence of COD (chapter 5, tables 5 and 6). However, Heuer *et al*. (1999) concluded in a study of 16 commercial dairy herds that a milk fat:protein ratio > 1.5 on the first test day increased the risk of COD. A difference between the two studies is that Heuer *et al.* used data for only the first milk recording, whereas we used data for two milk recordings. In a study by Zulu *et al.* (2002), a sharp increase in serum IGF-I occurred concurrently with the diagnosis of ovarian cysts in early lactation (16-33 days *post partum*, n=5). Moreover, from week 6 -10 *post partum* the serum IGF-I level was significantly higher in cystic cows than in normal cows. This is remarkable because circulating concentrations IGF-I decrease during periods of food deprivation (Breier *et al*., 1986; McGuire *et al*., 1995). Given that the mechanism underlying COD is not yet known, it is possible that the elevated IGF-I levels were the result rather than the cause of COD.

In our study of the results of treatment of COD (chapter 2), a substantial proportion of the diagnoses of COD were made 100 days or later *post partum*. Even though NEB was not measured directly, it would appear that many cows develop COD after the period of NEB. This would confirm the conclusion of another study with a different analytic approach (chapter 5) that the incidence of clinical COD is not related to a NEB. However, Britt (1992) suggested that a latent effect of postpartum metabolism on the quality of follicles, destined to be ovulated during the breeding period 60-80 days later, influences fertility by diminishing the viability of oocytes and by a lowering progesterone secretion by *corpora lutea*. This hypothesis was the result of a retrospective analysis of data classified solely by change in body condition scores (BCS) between week 1 and 5 *post partum*. However, Heuer (2000) considered BCS a poor diagnostic tool for detecting NEB (sensitivity 19-28%). Because our study (chapter 5), like the study of Britt (1992), was based on proxy parameters of NEB assessed from early lactation, it seems unlikely that the hypothesis of Britt is involved in the pathogenesis of COD. It might be interesting to compare the results of our study involving two milk recordings with the parameters Y_{max} en t_{max} with an analysis based on the first milk recording only.

In our study of the influence of NEB on reproductive performance after treatment (Chapter 6), the "hazard" for insemination after treatment was correlated with the proxy parameter for NEB, Δfatg. The conclusion was that an increased Δfatg in group 1 (1^{st} milk recording 0-9 days *post partum*) increased the "hazard" for insemination, but an increased Δfatg in group 3 (1^{st} milk recording 20-29 days *post partum*) decreased the "hazard" for insemination. Comparing these results with the results of treatment when day 60 *post partum* was used as cut-off between treatment groups (chapter 1), we conclude that NEB did not influence the recovery from COD of cows in which COD was diagnosed 60 days or later *post partum*. However, the "hazard" for conception in all groups was reduced with increasing Δfatg, though this effect was not significant in any of the groups. Nevertheless, it is possible that in individual herds with a severe NEB the energy imbalance may effect the conception rate. In this context, the hypothesis of Britt (1992) might be interesting. In particular, the decreased conception rate of cows in the low BCS group (higher conditioned cows at calving) in that study was remarkable.

Further investigations, particularly accurate measurement of NEB, are recommended and might lead to the conclusion that NEB influences conception after treatment of COD. Moreover, other health disorders, such as (sub)acute rumen acidosis or lameness, may directly or indirectly interfere with causative factors of COD. To carry out reliable investigations, an adequate registration of all health disorders at a herd level is necessary, with exact definitions of all diagnoses involved.

4. Conclusions

Briefly stated, the main results of the studies described in this thesis are:

- treatment of cows with clinical COD with GnRH after 30 days *post partum* is effective regardless of the day of diagnosis and treatment *post partum*;
- COD prolongs the intervals between calving and conception and between calving and 1^{st} insemination in dairy cows;
- treatment of cows with COD with GnRH does not increase the interval between insemination after treatment and conception;
- the estimated heritability of COD indicates a genetic predisposition in Dutch Black and White dairy cows;
- genetic correlations exist between milk production traits and COD;

- average maximum milk fat:protein ratio is not related with COD;
- changes in milk fat percentage and milk fat yield between the first and second milk recordings *post partum*, which are indicative of NEB, are not related with COD;
- a short interval between calving and NEB nadir in cows treated for COD with GnRH results in a shorter interval between treatment and insemination compared with cows with NEB nadir later in lactation;
- the moment of NEB nadir *post partum* does not significantly influence the interval between treatment with GnRH and conception in dairy cows with COD.

These results might prompt veterinary practitioners to improve the monthly monitoring of dairy herds, especially because the incidence of COD will probably increase in the future as a result of the genetic background of cows and the correlation between milk production traits and COD. Working according to a standard protocol is a prerequisite. While GnRH has proved to be a good choice for treatment of COD in dairy cows, veterinarians do need to monitor cows during treatment.

The underlying mechanisms of COD are still unclear; however, results indicate that, regarding the heritability of COD and the correlation between COD and milk production traits, molecular studies should be performed, particularly integrated studies of COD and metabolic hormones, associated with milk production traits. Given the fact that a causative role of NEB in the pathogenesis of COD seems unlikely, other mechanisms may exist by which metabolic and/or reproductive hormones affect the hypothalamo-pituitary-gonadal axis to cause COD.

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Chapter 8

Summary

Summary

In this thesis three main aspects of cystic ovarian disease (COD) in dairy cattle were studied:

- the results of treatment with gonadorelin[7] (GnRH) in relation to the day of treatment *post partum*, and reproductive performance after treatment;
- the heritability of COD and estimation of the genetic and phenotypic relationships with milk production traits;
- the role of the negative energy balance (NEB) *post partum* regarding the incidence of clinical COD and reproductive performance after treatment.

COD was defined as the persistence of an anovulatory follicular structure on one or both ovaries with a diameter of more than 2.5 cm in the absence of a corpus luteum, resulting in aberrant reproductive performance. All longitudinal studies were based on the same study design and standard fertility examination protocol (chapter 1), carried out by one veterinary practice[8] during ten years starting in june 1987.

1. Treatment with GnRH

In the first study the treatment results for two groups of cows diagnosed and treated for COD were evaluated (chapter 2). In total, 765 lactating dairy cows in 869 lactations were treated with 500 µg GnRH. Two treatment groups were formed (n=349 and n=520), based on the interval between calving and 1st treatment, with day 60 being the cut-off between the two groups. Cows were treated from 30 days *post partum*. Recovery after treatment of clinical COD was considered to be present when at least one of the following criteria were met during a subsequent examination:

- a *corpus luteum* and/or follicle < 2.5 cm on one or both ovaries;
- disappearance of the cyst without further palpable structures on the ovaries;
- calving as a result of insemination after treatment, in those cases lacking further information;
- no diagnosis of recurrence during the same lactation ("palpable" corpus luteum does not necessarily mean that endocrine regulation has recovered).

In group 1, 90.0% of first treatments were effective and in group 2 93.3% (p=0.08). This was reflected by the slightly higher number of treatments needed for animals in

[7] Fertagyl® Janssen-Cilag (originally Akzo-Intervet)

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group 1 (1.11 versus 1.07, P=0.08). The efficacy rate (number of inseminations per conception) after 1st treatment did not differ significantly (1.63 versus 1.69, P=0.40), nor did the interval between 1st insemination after treatment and conception (P<0.63). In conclusion, it can be stated that gonadorelin is effective as therapy for COD irrespective of the time of diagnosis and treatment.

In a subsequent study (chapter 3), the reproductive performance of dairy cows with COD, treated with 500 µg GnRH from 30 days *post partum* (n=925), was compared with that of unaffected cows (n=13,869). In both groups, 90.9% and 93.7% of the cows conceived (P=0.053). The intervals between calving and 1st insemination and between calving and conception differed significantly between the groups (P<0.001), but the interval between 1st insemination and conception did not (P=0.31). The conception rate after 1st insemination and the number of services per conception were significantly higher in the COD group (P=0.001 and P=0.008, respectively). This was not surprising because all services before the diagnosis COD were included in the calculations. The conclusion was that a prolonged interval between calving and 1st insemination in cows with COD was responsible for the delayed interval between calving and conception. Thus, regular fertility monitoring can minimize the number of days open of cows with COD because treatment with GnRH does not result in a prolonged insemination-to-conception interval.

2. Heritability

In the third study (chapter 4), the heritability of COD in Dutch Black and White dairy cattle was investigated in combination with estimation of the genetic and phenotypic relation with milk production traits. Dairy cattle breeding programmes are largely focused on milk production traits and thus knowledge of the genetic correlation between these traits and COD is important in order to be able to predict COD incidence when following this breeding strategy.

In the data set used, the overall incidence of COD was 7.7% (1204 COD diagnoses from 15,562 lactations), with the lowest incidence in parity 1 and the highest in parity 3^+ cows. The farm incidence varied between 1.9% and 11.3%. The trait COD was considered as a binary expression (present/absent) of an underlying variable that is influenced by genetic and environmental factors. The genetic analysis was based on this threshold model, assuming an unobservable continuously distributed variable underlying COD, called the liability. The liability is related to the incidence of COD. As soon as the underlying variable exceeds a certain threshold, then the event (COD) is occurs. The estimated heritability on the underlying and observable scale was 0.10 and 0.09, respectively. The average breeding values for COD of all cows per year of calving, based on the heritability on the underlying scale, increased from -0.01 in 1987 to 0.20 in 1996, indicating a genetic trend in the population. The genetic correlation between COD and 305-day milk, fat, and protein yields was 0.35, 0.38, and 0.44, respectively. Thus, there is a genetic predisposition for COD in Dutch HF crossbreds. The genetic correlation between COD and milk yield indicates that ongoing breeding programmes selecting for milk production will increase the incidence of COD.

3. Negative Energy Balance

Two studies were carried out to investigate a possible relation between milk production parameters in early lactation and the incidence of COD and reproductive performance after treatment with GnRH of cows with COD, with special emphasis on the negative energy balance (NEB). The data set consisted of 6,911 lactations for 3,891 cows from 29 herds including 600 cases of COD (8.7%). The analysis was based on data for the first and second milk recordings *post partum.* However, in field studies there is considerable variation in when the first and second milk recording take place after calving, and in the curve of milk fat and protein content after calving. Therefore, cows were divided into four groups depending on the day of first milk recording. For each cow the peak milk yield (Y_{max}) after calving and the day in which the predicted peak milk yield (t_{max}) was reached were calculated. Daily changes in the average milk fat and protein percentage (Δfat, Δpro), and in milk fat and protein yield (Δfatg, Δprog) between both milk recording dates were calculated. They were used as proxy parameters for NEB, because in our studies we did not measure the energy balance of the peri-parturient or *post partum* cow directly.

In the fourth study (chapter 5), lactations in the COD group had a higher average maximum milk yield and energy corrected milk, and milk fat and protein content than lactations in the non-COD group. The average maximum milk fat:protein ratio did not differ significantly between the two groups. No significant relationship was found between changes in milk fat and protein percentage and milk fat and protein yield, and the incidence of COD. Thus, an increased peak milk yield and milk fat and protein content in early lactation are risk factors for the incidence of COD, but the average milk fat:protein ratio is not. Changes in milk fat percentage and milk fat yield, indicative for the presence of NEB, were not correlated with the incidence of COD in cows early in lactation.

The fifth study (chapter 6) was focused on the reproductive performance of cows with clinical COD after treatment with GnRH in relation to the presence of NEB in the early lactation period. Reproductive performance after treatment was assessed from the interval between treatment and 1st insemination (ITFI) and the interval between treatment and conception (ITC). On the basis of the daily change in milk fat yield (Δfatg) between the first and second milk recordings *post partum*, cows were considered to have passed the NEB nadir (positive Δfatg) or not (negative Δfatg). The effect of Δfatg was significant for group 1 and group 3 (1st milk recording on 0 to 9 days and 20 to 29 days p.p., respectively) with an increased and a decreased probability of being inseminated the first day after treatment (ITFI), respectively. However, no significant interaction was found between Δfatg and the groups with regard to ITC. Thus, NEB nadir early in lactation increases the likelihood of insemination after treatment, but the moment of the postpartum NEB nadir, early or late in lactation, does not influence conception. The net result is that cows with clinical COD and treated with GnRH that have a NEB nadir early in lactation have a 3-7 day shorter day-open interval than cows with a NEB nadir 10 to 20 days later.

Briefly stated, the main results of the studies described in this thesis are:

- treatment of cows with clinical COD with GnRH after 30 days *post partum* is effective regardless of the day of diagnosis and treatment *post partum*;
- COD prolongs the intervals between calving and conception and between calving and 1st insemination in dairy cows;
- treatment of cows with COD with GnRH does not increase the interval between insemination after treatment and conception;
- the estimated heritability of COD indicates a genetic predisposition in Dutch Black and White dairy cows;
- genetic correlations exist between milk production traits and COD;
- average maximum milk fat:protein ratio is not related with COD;
- changes in milk fat percentage and milk fat yield between the first and second milk recordings *post partum*, which are indicative of NEB, are not related with COD;
- a short interval between calving and NEB nadir in cows treated for COD with GnRH results in a shorter interval between treatment and insemination compared with cows with NEB nadir later in lactation;
- the moment of NEB nadir *post partum* does not significantly influence the interval between treatment with GnRH and conception in dairy cows with COD.

Samenvatting

In dit proefschrift worden drie belangrijke aspecten van cysteus ovariële follikels (COF) bij melkkoeien behandeld:

- de resultaten van behandeling met gonadorelin[9] gerelateerd aan de dag van behandeling *post partum*, en analyse van een aantal vruchtbaarheidskengetallen van behandelde koeien in vergelijking met een groep koeien zonder diagnose COF;
- de erfelijkheid van COF en een schatting van de genetische en phenotypische correlatie met melkproductiekenmerken;
- de vraag of de incidentie van COF en de vruchtbaarheid na behandeling beïnvloed wordt door de negatieve energiebalans (NEB) *post partum*.

COF werd gedefinieerd als het persisteren van een anovulatoire structuur op één of beide ovaria met een diameter van meer dan 2.5 cm zonder dat een *corpus luteum* aanwezig is, met als gevolg afwijkende vruchtbaarheidskengetallen. De opzet van alle longitudinale onderzoeken was gelijk, gebaseerd op een standaard onderzoeksprotocol (hoofdstuk 1), en werden vanaf juni 1987 gedurende 10 jaar uitgevoerd in één dierenartsenpraktijk[10].

1. Behandeling met GnRH

In het eerste onderzoek (hoofdstuk 2) werden de resultaten geanalyseerd van twee groepen koeien met COF na behandeling met 500 µg GnRH. In de ene groep (n=349) had de diagnose en behandeling plaats tussen 30 en 59 dagen, en in de andere groep (n=520) vanaf 60 dagen *post partum*. De behandeling werd als succesvol beschouwd als gedurende een vervolgonderzoek tenminste één van de volgende criteria op een koe van toepassing was:

- aanwezigheid van een *corpus luteum* en/of een follikel < 2.5 cm op één of beide ovaria;
- afwezigheid van een cyste zonder duidelijk te palperen structuren op de ovaria;
- geboorte van een kalf als resultaat van een inseminatie na behandeling, in gevallen waarbij geen vervolgonderzoek werd ingesteld na behandeling;

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[9] Fertagyl® Janssen-Cilag (originally Akzo-Intervet)

[10] Dierenartsenpraktijk Mid-Fryslân, Hopmanshof 1, 8491 BK Akkrum, Nederland

- geen recidief tijdens dezelfde lactatie, van belang omdat een “palpabel” *corpus luteum* niet hoeft in te houden dat endocrinologisch volledig herstel is opgetreden.

Er was geen significant verschil tussen beide groepen noch wat betreft het succes van de behandeling (90.0% resp. 93.3%, p=0.08), noch het aantal behandelingen (1.11 versus 1.07, p=0.08). Het efficiëntiegetal (= aantal inseminaties per dracht) na 1e behandeling verschilde niet significant (1.63 versus 1.69, p=0.40), evenmin als het interval 1e inseminatie na behandeling–conceptie (p<0.63). Concluderend kan gesteld worden, dat gonadorelin, ongeacht het lactatiestadium, uitstekend werkzaam is als behandeling van melkkoeien met COF.

In een volgend onderzoek (hoofdstuk 3) werden vruchtbaarheidskengetallen van melkkoeien met COF (n=925), die vanaf 30 dagen *post partum* behandeld waren met 500 µg GnRH, vergeleken met die van koeien zonder COF (n=13,869).

Uiteindelijk bleek in de multivariate analyse (na correctie voor bedrijfseffecten, interval partus–1e inseminatie, pariteit en jaar van afkalven), dat in de COF groep 90.9% en in de groep zonder COF 93.7% (p=0.053) drachtig was geworden. De intervallen partus-1e inseminatie en partus-conceptie verschilden significant (p<0.001), maar het interval 1e inseminatie-conceptie niet (p=0.31).

Het drachtigheidspercentage na 1e inseminatie en het efficiëntiegetal (=aantal inseminaties per drachtigheid) waren significant hoger in de COF groep (p=0.001 resp. p=0.008). Dit lag voor de hand omdat in deze kengetallen ook de inseminaties meetelden die uitgevoerd werden voordat de diagnose COF gesteld werd.

Uit deze resultaten kan de conclusie worden getrokken dat een verlengd interval partus-conceptie bij koeien met COF volledig het gevolg is van een langer interval partus-1e inseminatie. Regelmatige vruchtbaarheidsbegeleiding op melkveebedrijven zal de tussenkalftijd van koeien met COF kunnen beperken, omdat deze dieren na behandeling met GnRH geen langer inseminatie-conceptie interval behoeven te hebben dan dieren zonder COF.

2. Erfelijkheid

Productiekenmerken (melkgift, melkvet en -eiwit) zijn in de melkveehouderij door de jaren heen de belangrijkste selectiecriteria geweest. Het is van belang inzicht te hebben in de genetische correlaties tussen productiekenmerken en COF, met name om enigszins een voorspelling te kunnen doen van de gevolgen die selectie op productiekenmerken kan hebben op de incidentie van COF.

Door ons werd van 1987 – 1996 een onderzoek (hoofdstuk 4) gedaan naar de erfelijkheid van COF bij de Nederlandse zwartbonte melkveestapel. Daarnaast werd getracht een schatting te maken van de genetische en phenotypische relatie van COF met productiekenmerken.

De incidentie van COF van alle koeien in de dataset was 7.7% (1204 diagnoses COF bij 15,562 lactaties). De laagste incidentie kwam voor bij vaarzen, terwijl de koeien met pariteit ≥ 3 de hoogste incidentie hadden. De incidentie per bedrijf varieerde van 1.9% to 11.3%. Bij de analyse werd er van uitgegaan dat COF een binaire expressie (aanwezig/afwezig) is van een onderliggende latente variabele ("liability"), die beïnvloed wordt door genetische en omgevingsfactoren. Op dit zogenaamde drempelmodel werd de genetische analyse gebaseerd. De "liability" is gerelateerd aan de incidentie. Zodra de "liability" een bepaalde grens overschrijdt dan is er sprake van COF. De niet waarneembare erfelijkheidsgraad ("heritability on the underlying scale"), geschat op basis van het drempelmodel, bedroeg 0.10. Deze werd vervolgens omgerekend naar de waarneembare erfelijkheidsgraad ("heritability on the observable scale") en kwam uit op 0.09. Gebaseerd op de niet waarneembare erfelijkheidsgraad nam de gemiddelde fokwaarde van alle koeien van 1987 tot 1996 toe van -0.01 tot 0.20. Dit betekent dat er een genetische trend aanwezig is in de populatie. De genetische correlatie van COF met 305-dagen melkproductie, vet- en eiwitgrammen was respectievelijk 0.35, 0.38 en 0.44. Er kan geconcludeerd worden dat er een genetische predispositie bestaat voor COF in de Nederlandse zwartbonte melkveehouderij. Het feit, dat genetische correlaties van COF met productiekenmerken zijn aangetoond, betekent dat de incidentie van COF zal blijven toenemen als de prioriteit van de selectiecriteria niet wordt verlegd.

3. Negatieve energiebalans

In het vierde en vijfde onderzoek (hoofdstuk 5 en 6) stond de relatie tussen COF en de negatieve energiebalans (NEB) centraal. In het ene onderzoek ging het om de vraag of de incidentie van COD beïnvloed wordt door de NEB, het andere betrof de vraag of de vruchtbaarheid van koeien na behandeling met GnRH in positieve of negatieve zin gerelateerd is aan de NEB.

De dataset bestond uit 6,911 lactaties (3891 koeien) op 29 bedrijven inclusief 600 gevallen van COF (8.7%). De analyse was gebaseerd op de uitkomsten van de eerste twee melkcontroles *post partum.* In een veldonderzoek verschillen per individuele koe de lactatiestadia, waarop de melkcontroles plaatsvinden. Bovendien heeft elke koe een eigen lactatiecurve voor melkproductie, melkvet en eiwit. Daarom werden, afhankelijk van het tijdstip van de eerste melkcontrole, de koeien verdeeld in vier groepen (hoofdstuk 7, figuur 1). Van elke koe werd de piekproductie van melk, melkvet en -eiwit bepaald (Y_{max}). Verder werd bij benadering de dag berekend, waarop de piekproductie werd bereikt (t_{max}). De dagelijkse toe- of afname van het gemiddelde vet- en eiwitpercentage (Δfat, Δpro) en van de gemiddelde vet- en eiwitgrammen (Δfatg, Δprog) tussen beide melkcontroledatums werd vastgesteld. De verkregen waarden werden vervolgens gebruikt als proxyparameter voor NEB, omdat in deze onderzoeken NEB niet nauwkeurig werd gemeten.

De resultaten van het vierde onderzoek toonden aan, dat lactaties in de COF-groep hogere gemiddelde piekproducties van melk, meetmelk, melkvet en -eiwit hadden dan lactaties zonder COF, maar dat de gemiddelde vet/eiwit verhouding niet verschilde tussen beide groepen. Er werd bovendien geen correlatie gevonden tussen enerzijds de dagelijkse toe- of afname van melkvet en -eiwitpercentage en van melkvet en -eiwitgrammen en anderzijds de incidentie van COD.

De conclusie van dit onderzoek was, dat een hogere piekproductie melk, melkvet en -eiwit in het begin van de lactatie de kans op COF verhoogt. De vet/eiwit verhouding in de melk en de gebruikte proxyparameters voor NEB hadden geen relatie met het vòòrkomen van COF.

Het vijfde onderzoek was er op gericht na te gaan of NEB de vruchtbaarheid van koeien met COF, behandeld met GnRH, beïnvloedt. De vruchtbaarheid werd beoordeeld aan de hand van twee intervallen, namelijk behandeling-1e inseminatie (ITFI) en behandeling-conceptie (ITC). NEB werd, evenals bij bovengenoemd onderzoek, benaderd via de proxyparameter Δfatg. Er werd van uitgegaan dat een koe de NEB nadir gepasseerd was bij een positieve Δfatg, terwijl dat bij een negatieve waarde nog niet het geval was. Het effect van Δfatg was significant voor de groepen 1 en 3 (1e melkcontrole op respectievelijk 0-9 en 20-29 dagen), met een toenemende respectievelijk een afnemende kans om de dag na behandeling te worden geïnsemineerd. Er is echter in geen van de groepen een significant effect aangetoond van Δfatg op ITC. Dit leidde tot de conclusie dat een NEB nadir vroeg in de lactatie de kans op inseminatie bevordert, maar dat de kans op conceptie door NEB nadir niet wordt beïnvloedt. Uiteindelijk hadden met GnRH behandelde koeien met COF, met een NEB nadir vroeg in de lactatie, een 3-7 dagen kortere tussenkalftijd dan koeien waarbij de NEB nadir 10-20 dagen later lag.

Curriculum vitae

Gerrit Aart Hooijer werd geboren op 29 maart 1953 in Apeldoorn. In 1971 behaalde hij op het Christelijk Lyceum te Apeldoorn het diploma Gymnasium-α. Na een overbruggingsjaar om lacunes in de kennis van de exacte vakken aan te vullen (de zgn. "cursus Blauw") begon hij in 1972 de studie diergeneeskunde, die hij in februari 1979 afrondde met het behalen van het dierenartsdiploma. De eerste werkkring was Dierenartsenpraktijk "de Beuk" in Wolvega, waar hij werkzaam was tot oktober 1979. Toen verruilde hij Wolvega voor de Dierenartsencombinatie Akkrum-Oldeboorn te Akkrum, de huidige Dierenartsenpraktijk Mid-Fryslân. Zijn werkzaamheden bestaan, naast de eerstelijnsdiergeneeskunde voor meerdere diersoorten, voornamelijk uit de rundergezondheidszorg. Met name de bedrijfsbegeleiding- en advisering neemt een groot deel van de tijd in beslag. Daarnaast participeerde hij in diverse studiegroepprojecten, zoals "Meten is weten", PGR en Satellietproject. In 1999 werd hij erkend als specialist rundergezondheidszorg, waarna hij in 2000 H^3 Consultancy oprichtte, een consultancybureau voor de tweedelijns rundergezondheidszorg. Binnen de Koninklijke Nederlandse Maatschappij voor Diergeneeskunde heeft hij verschillende functies bekleed, zoals voorzitter van de afdeling Friesland, lid van het Algemeen Bestuur, en bestuurslid van de Groep Rund.