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Chapter 6 General Discussion

Ovarian cysts are a cause of reproductive failure and economic loss in postpartum dairy cows. Ovarian cysts are often formed during the period of ovarian rebound (e.g. at the time of expected first postpartum ovulation) but can spontaneously resolve. However, ovarian cysts can often reoccur and persist which will delay time to first service and reduce the number of service opportunities and thus increase the calving to conception interval. Although the exact mechanisms of cyst development are still unclear it is most likely that cysts are caused by a failure of the positive and negative feedback mechanisms which regulate the HPO axis. However, additional ovarian factors are likely to play an important modulatory role. Moreover, given the impact of ovarian cysts on the dairy industry, there is also a need to determine the risk factors (e.g., nutritional) associated with cyst development and to assess the efficacy of current veterinary methods of diagnosis. Using a unique combination of research, this thesis has investigated problems at diagnosis, implications of cow management and mechanisms of cyst formation.

The main findings of this thesis are summarised as follows;

- ❖ Despite the widespread use of ultrasound and availability of on-farm progesterone tests, the accurate diagnosis of ovarian cyst type still remains a real challenge and continues to hinder efficient therapeutic treatment.
- ❖ Determination of milk progesterone concentrations is a valuable tool that should form an integral component of the veterinary diagnosis when assessing ovarian cyst diagnosis, and to monitor the efficacy of a chosen treatment. Furthermore, the routine veterinary use of a cow-side milk

progesterone test may be effective in reducing the number of days open postpartum.

- ❖ All cows during the management trial had a period of NEB at calving which was sustained in some, but not all, cows. A subset of this population (35.6%) developed an ovarian cyst at some point postpartum. Cows with an ovarian cyst (FC or LC) exhibited a different metabolic profile to those without at different time points; prior, during and after calving. This was associated with elevated BHB, albumin and triglyceride concentrations ($P < 0.05$), lower total protein concentrations and lower GGT activities ($P < 0.05$). Furthermore these changes were most noted to occur from the beginning of the dry period up until 5 weeks postpartum.
- ❖ Negative energy balance occurs earlier in twin pregnancies than in singletons and this manifested in an earlier elevation of NEFA and BHB concentrations and loss of BCS. Consequently, nutritional monitoring may be used to detect and manage twin pregnancies so that the incidence of twin pregnancy associated problems (dystocia, interval to oestrus, interval to pregnancy, etc.) is reduced.
- ❖ Down-regulation of the HPO axis using a GnRH agonist protocol followed by ovarian stimulation lead to the formation of aberrant pre-ovulatory follicles, and 50% of heifers failed to exhibit an LH surge within 120h of luteal regression.
- ❖ All heifers, irrespective of LH surge, demonstrated great variation in follicle size as well as aberrant steroidogenesis (e.g. high intra-follicular

concentrations of oestradiol, progesterone and androstenedione as well as the absence of steroidogenic enzymes).

6.1 Clinical relevance of these findings

The findings in this thesis are potentially clinically important in the veterinary diagnosis and treatment of bovine ovarian cysts. This thesis has identified the importance of an oversight during the veterinary diagnosis of ovarian cysts, identified potential risk factors for the development of ovarian cysts, and characterised the use of a GnRH agonist protocol in providing a model for ovarian cyst formation that, with some development, will aid the future study of ovarian cysts.

Data presented in the diagnostic chapter has highlighted the weakness of using just rectal palpation and/or transrectal ultrasound in the diagnosis of ovarian cysts. Furthermore, this data has demonstrated that the incorporation of milk progesterone testing with rectal palpation and/or transrectal ultrasound may be beneficial in the diagnosis of ovarian cysts. Moreover, this data suggests a possible reduction in the number of days open when hormonal and ultrasonographic diagnosis agreed, however, this difference was not significant. It is concluded therefore that progesterone assays should be introduced as part of a gold standard method for diagnosing and differentiating between cyst types. There are at least three companies that distribute easy-to-use progesterone detecting kits (Ridgeway Science (Gloucestershire, UK), Target (New Jersey, USA) and Accufirm (Maine, USA)), and all these kits come with comprehensive instructions. Importantly, these assays can be performed quickly with results that are clear to interpret, so they could be easily executed in the farm environment. A cost-benefit analysis of the use of milk

progesterone tests on the farm has been published. In this study, the authors report that the use of progesterone detecting kits was successful in differentiating the cyst types (based on high or low progesterone), but that the expense of the kit and labour was economically unjustifiable (Ruiz *et al.*, 1992). However, since this analysis has been done a new, more sensitive cow-side progesterone test has been developed. Based on the Ridgeway progesterone ELISA (Ridgeway Science, Gloucestershire, UK), this new cow-side test has been modified to contain a lower concentration of antibodies, thus increasing the sensitivity of the assay and reducing its cost (MDC Final Report, 2006). Furthermore, Ruiz *et al.*, (1992) assumed a voluntary waiting period of 40 days whereas in the UK, the voluntary waiting period is between 60-65 days. While treating cysts early in lactation (≤ 49 days postpartum) can be effective (López-Gatiús *et al.*, 2002), these cysts will often spontaneously resolve. Moreover, a study carried out in Spain examined a number of reproductive traits, including ovarian cysts, and demonstrated that dairy cow fertility has declined over a 10 year period (López-Gatiús, 2003). With respect to UK dairy herds, published studies generally focus on data collected from commercial herd records prior to 1998 (McGuirk, 2004). However, a more recent publication indicates that dairy cow fertility in the UK has further declined between 2000 and 2006 (Hudson *et al.*, 2010). Despite an unfavourable review of the economics of on farm milk progesterone testing, Ruiz *et al.*, (1992) also reported some beneficial results. The authors demonstrated a decrease in the number of days open and an overall increase in reproductive performance (Ruiz *et al.*, 1992). Similarly, Sprecher *et al.*, (1988) and Bajema *et al.*, (1994) also demonstrated a decrease in days open as well as an increase in net return per cow. These results combined provide evidence that

determination of progesterone on farm can be a justifiable expense, potentially dependent on herd size and the extent of fertility problems observed.

Further evidence for the potential value of routine progesterone measurements is provided by the study of Simersky and colleagues who developed a sensitive on farm progesterone assay to assist with the rapid detection of the success or failure of artificial insemination (Simersky *et al.*, 2007). Moreover, in order to monitor luteal activity Claycomb *et al.*, (1998) proposed and developed a progesterone assay that could be used in conjunction with a biosensor. This biosensor would be attached to the milking machine and would automatically determine milk progesterone concentrations. Once detected, progesterone data would be available to the farmer either via download from the milking machine or automatic upload onto a central farm recording system. Although both systems were not developed for use in the diagnosis and differentiation of ovarian cysts, they do have potential to be used in this area, either by retrospective analysis of the progesterone concentrations when an ovarian cyst is suspected, or in support of a veterinary diagnosis on farm.

In the management chapter, metabolic predisposing factors for ovarian cysts were retrospectively investigated. All of the cows involved in the trial (n=85) were in a state of NEB, and 35.6% developed an ovarian cyst. This is higher than expected since ovarian cysts typically occur at 6 to 19% (Kesler & Garverick, 1982). These data indicate that there was considerable individuality in how each cows' reproductive system responded to the metabolic challenge experienced during the transition period. This evidence suggests that sustained NEB cannot be defined as a specific causal factor of ovarian cysts but that certain alterations of metabolic parameters (specifically total protein, globulin, albumin, and triglyceride

concentration as well as GGT activity) may indicate that the liver is not functioning correctly which may, in turn, increase the risk of developing a cyst. Several studies have investigated the effect of NEB on reproductive performance in the postpartum period (Villa-Godoy *et al.*, 1988; Zulu *et al.*, 2002; Jorritsma, *et al.*, 2003). However, the measurement of specific metabolites (namely total protein, albumin, globulin, triglycerides and GGT) during the dry period may provide a means of monitoring liver function. This would allow the farmer to treat the liver problem nutritionally and inadvertently reduce the incidence of ovarian cysts. Evidence presented in this thesis supporting this theory comes from the identification of metabolic parameters traditionally measured to determine the health and functional capacity of the liver. Concentrations of total protein, globulin and GGT activities were decreased, and concentrations of triglyceride and albumin were increased, in cows with a cyst *vs.* cows without, although only triglyceride concentrations were elevated outside of normal expected values. This may indicate that liver function in cows with a cyst is altered *vs.* cows without a cyst, and warrants further investigation. Furthermore, this would enable a more effective dietary regime during the transition period and early lactation that may provide a more cost effective alternative to current therapeutic treatments. However, more work is required to define specific metabolic parameters of interest and to potentially relate these to severity of NEB and the formation of ovarian cysts.

In this thesis, the mean body condition scores of cows that developed luteal (n=10) and follicular (n=21) cysts were 4 and 3.5 respectively, and showed no difference to no-cyst cows (n=48: BCS=3.5) at the beginning of the dry period. It has been reported that cows that were over conditioned (>3.5 BCS) during the dry period and at calving experienced greater loss of BCS postpartum, they also had a

deeper and longer NEB period, poor liver function and low circulating IGF-I concentrations (Zulu *et al.*, 2002), all of which were associated with cystic ovaries, persistent corpora lutea and inactive ovaries (Zulu *et al.*, 2002). However, data presented in this thesis was inconclusive with regard to over conditioning at the start of the transition period and the occurrence of ovarian cysts.

Feeding increased levels of energy and protein during the dry period can result in a decreased incidence of both metabolic and reproductive disorders postpartum (Curtis *et al.*, 1985). Lucy *et al.*, (1991) demonstrated an effect of diet on the distribution of follicles within each size class rather than the overall number of follicles present, and stated that the effect of diet was limited to the early postpartum period, (e.g. influencing the time taken till first ovulation). Furthermore, cows fed a high fat diet during the dry period demonstrated a significant alteration in hepatic lipid metabolism immediately post-calving (Grum *et al.*, 1996), resulting in decreased accumulation of triglycerides within hepatic cells and decreased plasma NEFA concentrations by day one postpartum, without affecting BCS. Therefore, if there is an association between NEB and ovarian cysts, an alteration in the inclusion of fats, proteins and energy may help alleviate this problem. During the management trial reported in this thesis, it was revealed that the ration fed to the cows was not the exact ration formulated by the nutritionist; this may have contributed to the development of the reproductive disorders, including ovarian cysts, which were observed postpartum in these cows. The decision to alter the ration was made by a farm labourer and not the farm owner, who was not aware of this change. As herd sizes increase, it is not uncommon for farm owners to employ labourers to assist them in daily animal husbandry chores. These labourers may be less invested in the maximising milk production from the cows and therefore may be

more likely to rely on their own work experience with regards to ration formulations, thus this may result in cows that are not underfed, but that may be receiving inadequate nutrition to support them through the transition period. Despite this finding, this trial was focussed on the impact of NEB on the development of ovarian cysts, irrespective of ration formulation.

Some published studies indicate that the content of the ration fed to postpartum cows may require further investigation; cows with a milk fat:protein ratio early postpartum >1.5 had a higher risk of ketosis, displaced abomasum, ovarian cysts and lameness (Heuer *et al.*, 1998). The same study also reported that high yielding cows in early lactation were also at direct risk of ovarian cysts and lameness, demonstrating a negative correlation between milk yield early postpartum and subsequent reproductive performance (Heuer *et al.*, 1998). High yielding cows with low concentrations of insulin were also more likely to develop an ovarian cyst (Vanholder *et al.*, 2005), and the authors stated that these cysts arose solely from the low insulin concentrations and were not associated with any other hormonal or metabolic alterations. The results from this thesis found that there was no correlation between high 305d milk yield and the occurrence of ovarian cysts however; although insulin concentrations were not determined it is likely that they were low as a result of sustained NEB (Roche *et al.*, 2000). Low insulin concentrations can result in decreased LH pulsatility which, in turn, can limit the capacity of the ovaries to respond to gonadotropins (Canfield & Butler, 1990), causing a delay in resumption of cyclicity. Based on the findings of Vanholder *et al.*, (2005), the measurement of insulin concentrations in the context of the management study may provide a potentially useful marker of ovarian cysts development.

Data collected from twin bearing cows in this thesis has identified an opportunity to improve the management of this group of animals. Metabolite concentrations indicate that cows bearing twins enter a period of NEB one week earlier than single bearing cows; this is likely due to the increased requirements on the cow in late gestation to facilitate twin fetal growth. An increased incidence of dystocia, retained placenta, as well as a longer postpartum interval to oestrus, longer interval to pregnancy, lower conception rates, and decreased birth weights, have all been reported in postpartum cows calving twins (Gregory *et al.*, 1990; Echtenkamp & Gregory, 1999). Extending the transition period to 8 weeks around calving in twin bearing cows, as suggested by Fricke (2011), and separation of cows expecting twins in the dry period may potentially alleviate some of these reported problems. As previously discussed, the identification of cows bearing twins would require no more than a thorough check of both uterine horns at PD; this would allow the stockmen to identify these cows at the beginning of the dry period and to separate them in order to cater for their metabolic needs. Supplementation of the diet with seed oils (e.g. α -linolenic acid), fermentable carbohydrates (e.g. starch) and trace elements, particularly zinc and selenium, has been shown to decrease postpartum lipolysis, decrease fetal loss, and facilitate cows in re-establishing a positive energy balance postpartum (Graham *et al.*, 1994; Grummer *et al.*, 1995; Huxley, 2004; Ambrose *et al.*, 2006; Silvestre *et al.*, 2007; Caldarri-Torres *et al.*, 2011). Experimentation of the level of inclusion of these elements into specific diets for cows bearing twins may facilitate their ability to adapt to the metabolic challenges they experience during the transition period. Furthermore, postpartum, the stockmen would be better able to identify those cows that are likely to require more veterinary attention in order to resume cyclicity and be successfully inseminated.

Data presented in the model for the study of ovarian cysts chapter have confirmed the use of the GnRH agonist protocol as an effective model for the study of ovarian cyst formation. Results from this thesis confirm that this model results in the formation of a number of large cyst-like structures that are likely formed from follicles that failed to ovulate. Furthermore, absent or reduced immunoexpression of steroidogenic enzymes (CYP17A1 & CYP19A1), observed in 12.5% of these structures, is indicative of aberrant steroidogenesis. With some development, this model could be useful in further investigations into the aetiology of ovarian cysts by allowing the inclusion and/or exclusion of experimental stressors or potential treatments, to examine the effect they may have on cyst formation. For example, this model could be used in conjunction with a variety of nutritional regimes to elucidate the impact of differing degrees of NEB on postpartum ovarian cyst development. Furthermore, since the diagnostic chapter indicated that current therapeutic treatments for ovarian cysts were sub-optimal, this model may be used to induce ovarian cysts and facilitate the development of improved therapeutic treatments. However, the extent to which the cysts arising from this model are comparable with naturally occurring cysts is unknown. The acquisition of a large quantity of reproductive tracts of cows with naturally occurring cysts from abattoirs would provide material for comparison of steroidogenic enzyme detection and intra-follicular steroid concentrations, providing a better insight into how comparable these cysts are.

6.2 Limitations of this work

Recruiting farmers to participate in this research was one of the first challenges encountered in this thesis. To achieve results that can be translated to farm practice

it was essential to use a large cohort of commercial dairy cows, providing a true reflection of the nutritional and environmental stressors experienced by cows in the commercial farming environment. As well as healthy cows, this cohort included those suffering with common metabolic and reproductive disorders, i.e. mastitis, NEB, ovarian cysts and endometritis. It was difficult to persuade farmers to participate in these studies as they were lengthy timeframes (months not weeks) and involved distracting the herdsmen from their day to day duties.

The likely incidence of ovarian cysts is difficult to predict from herd to herd and there is no way to accurately predict the frequency of follicular or luteal cysts. Recruiting large commercial herds is the only way to increase the likelihood that a sub-population of cows will develop ovarian cysts, those cows that do not develop a cyst would serve as a control group; however this can only be determined retrospectively. This problem may be overcome in research animals under the Animals in Scientific Procedures Act of 1986 (A(SP)A 86), using models such as the one outlined in the mechanistic post-buserelin study to induce ovarian cysts for study.

Recruiting veterinarians onto the diagnostic study of this thesis was challenging as they felt that their judgement was being called into question with regards to differentiating between cyst types and administration of chosen treatments. The few veterinarians that did choose to participate were those who had an interest in research and felt they knew the right farmers who would also be willing to participate in such a long period of observation.

6.3 Final Conclusions

The broad spectrum of research conducted for this thesis has examined ovarian cysts from both mechanistic and diagnostic angles. These results have identified problems at diagnosis, differentiating between cyst types, leading to ineffective treatment choices and indicate that progesterone, alongside conventional techniques, is a valuable tool that is currently being overlooked by practising veterinarians. Sustained NEB during the transition period can result in abnormal ovarian structures in a subset of postpartum cows. Furthermore, identification of some metabolic parameters may facilitate the management of liver function and thus inadvertently reduce the incidence of ovarian cysts. In addition, better cow management is needed to cope with the metabolic demands of both single and twin bearing cows during the transition period. Finally, this thesis has demonstrated that the treatment of heifers with a GnRH agonist protocol results in a crop of aberrantly steroidogenic cystic-like structures, making it a potential tool in the study of this common ovarian pathology.