Chapter 3 Diagnostic Study

It is common practice for veterinarians to diagnose ovarian cysts based on one visit to the affected animal, despite being taught to reassess a cyst diagnosis one week later (Wapenaar, W., personal communication; Huxley, J., personal communication). Unfortunately, this does not provide them with an accurate picture of the problem; one examination will only indicate the size of the potential cyst and the status of ovarian function on the contra-lateral ovary. It would not, though, indicate how long the potential cyst has been present on the ovary and without hormonal analysis, it is impossible to determine whether the potential cyst is steroidogenic or not. The aim of this study was to determine difficulties associated with ovarian cyst diagnosis and the subsequent effect on success of treatment outcome. An inaccurate cyst diagnosis can lead to inappropriate choice of treatment, potentially prolonging the life of the cyst and increasing the number of days open (i.e. not pregnant). Previously, work has demonstrated that 34% of cyst diagnosis disagreed with milk progesterone concentrations (n=155) (Kendall, Mann & Flint, unpublished data). This study looked to expand on that previous work to include looking at the efficacy of chosen treatments.

3.1 Materials and methods

3.1.1 On-farm protocol

Farmers were recruited onto this trial by participating veterinarians, chosen based on their willingness to comply with the research, as it involved a long period of sample collection. Veterinarians were asked to complete a proforma with information on

cyst diagnosis, parity and choice of treatment. No treatment instructions were given to the veterinarians other than to treat each cyst as they routinely would and include details on the proforma.

3.1.2 Milk collection

To effectively monitor efficacy of treatment, milk samples were collected once a week from diagnosis for 12 further weeks, encompassing potentially up to 4 oestrous cycles. Having agreed to participate in the trial, farmers were given a kit containing everything they would need to collect and return samples to the laboratory. Farmers were instructed to collect milk samples into 25ml universal tubes (Sterilin Ltd, Newport, UK) containing a broad spectrum milk preservation tablet (D&F Control Systems Inc., CA, USA), which were then mixed and decanted into 75x12mm polypropylene tubes (Sarstedt, Leicester, UK) prior to postage. The farmers were sent a return form to complete and post with the samples. The form was used to detail dates samples were taken, missed samples, any additional treatments and outcome of treatment (e.g. cyst resolved and/or successful service). Farmers were also telephoned after sampling was complete to determine outcome of the treatment (i.e. pregnant/not pregnant).

3.1.3 Milk progesterone enzyme-linked immune-sorbent assay (ELISA)

On arrival at the laboratory, samples were stored at 4°C and analysed together in batches. Samples were thoroughly mixed prior to the assay and were not stripped of fat. Milk progesterone levels were measured using pre-coated ELISA plates specific for bovine milk (Ridgeway Science, Gloucestershire, UK) and manufacturer's instructions were followed. The two QCs included into this ELISA were a low

(2ng/ml) and a high (8ng/ml) milk progesterone with an assay sensitivity of 1ng/ml. Inter-assay % CV were as follows; Low (2 ng/ml) QC 29.3%, and high (8ng/ml) QC 23.0%, and intra-assay % CV was calculated at <10%.

Milk progesterone ELISA procedure

Plates and reagents were removed from 4˚C storage and allowed to come to room temperature before commencing the assay. All wells were emptied and the plate was inverted and tapped on towel until dry. Unknown samples (10µl), standards (10µl) and QCs (10µl) were added to the plate in duplicate. The progesterone-enzyme label (200µl) was added to all wells and left to incubate for 1-1.5 hours at room temperature (18-20˚C). The photosensitive alkaline phosphatase substrate was reconstituted at this stage and left in a dark cupboard until required. Following incubation, wells were washed under a running tap. The previously reconstituted substrate was now added to each well (200µl per well) and allowed to incubate for 10-30 minutes. The absorbance were then determined at 570nm on a Multiskan Ascent 96/384 plate reader (MTX Lab Systems, Inc., Vienna, VA, USA).

3.2 Data analysis

To determine the agreement or disagreement between cyst diagnosis and milk progesterone levels, a cut-off point of 3ng/ml was chosen. Thus milk progesterone concentrations ≥3 ng/ml or <3 ng/ml was indicative of the presence or absence of luteal activity, respectively (Darwash *et al.,* 1997). Resumption of cyclicity was defined to have occurred when a pattern of two high followed by one low milk progesterone samples was observed during a three week window (Bajema *et al.,* 1994). Treatment was classified as successful if resumption of cyclicity had

occurred and/or an animal became pregnant within the 13 week study period, otherwise it was deemed unsuccessful. Significant associations between agreement of cyst diagnosis and treatment outcome were determined using a Fishers Exact test (Graphpad Software Inc., CA, USA). The effect of veterinary and/or hormone diagnosis on the time interval from cyst diagnosis to conception was determined using a two way ANOVA (Genstat 12, VSN International, Hemel Hempstead, UK).

3.3 Results

Seven veterinarians from four practices based in Dorset, Derbyshire and Shropshire recruited farmers from twelve different farms onto this study. In total, 30 cows were recruited onto the trial. Of the complete data (30 diagnosed cysts), veterinarian diagnosis differentiated these into 22 follicular and 8 luteal cysts, whereas hormonal diagnosis identified 16 follicular and 14 luteal cysts.

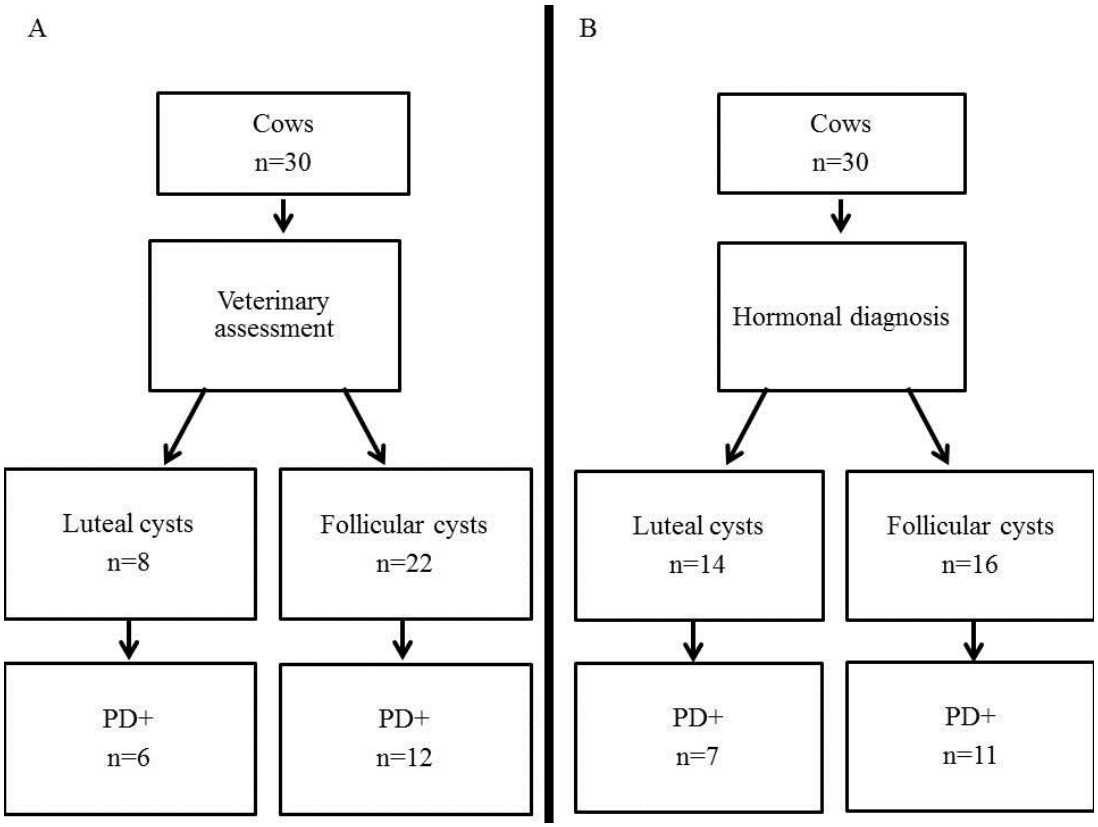

Figure 3.1: Number of follicular and luteal cysts identified by A) veterinary assessment or B) hormonal diagnosis; plus the subsequent number of cows from each group that successfully achieved pregnancy by the end of the 13 week observation period.

PD+ = pregnancy successfully detected.

3.3.1 Follicular cysts

3.3.1 1 Reported treatment regimes

Follicular cysts were treated with the following treatments; 1) Application of a PRID (1.55g progesterone; Ceva Animal Health, Buckinghamshire, UK) or CIDR (1.38g progesterone; Pfizer Ltd., Kent, UK) at diagnosis, for 14 days (n=3). 2) Intramuscular (im) injection (5mls) of the GnRH agonist buserelin acetate (0.004mg/ml) (Receptal, MSD Animal Health, Milton Keynes, UK), at diagnosis, followed 14 days later by injection (2ml,im) of the prostaglandin $F_{2\alpha}$ analogue, cloprostenol (250µg/ml) (Estrumate, MSD Animal Health, Milton Keynes, UK) (n=10). 3) Injection (5ml, im) of buserelin acetate (0.004mg/ml) (Receptal, MSD Animal Health, Milton Keynes, UK) at diagnosis (n=5). 4) A double dose (10mls, im) of buserelin acetate (0.004mg/ml) (Receptal, MSD Animal Health, Milton Keynes, UK) at diagnosis followed 14 days later by 2ml of the prostaglandin $F_{2\alpha}$ analogue, cloprostenol (250µg/ml) (Estrumate, MSD Animal Health, Milton Keynes, UK) (n=1). 5) Injection (2ml, im) of the prostaglandin $F_{2\alpha}$ analogue, cloprostenol (250µg/ml) (Estrumate, MSD Animal Health, Milton Keynes, UK) (n=1).

3.3.1.2 Parity and cysts

Of the cysts diagnosed as follicular, the majority (17/22; 77%) were observed in cows between parity 2 and 4. There was only 1 cyst diagnosed in a cow of parity 1, and 4 cysts diagnosed in cows of parity 5 or above (Figure 3.2). As these cows were not from one herd it is impossible to know the parity distribution of each herd from which these milk samples were collected from.

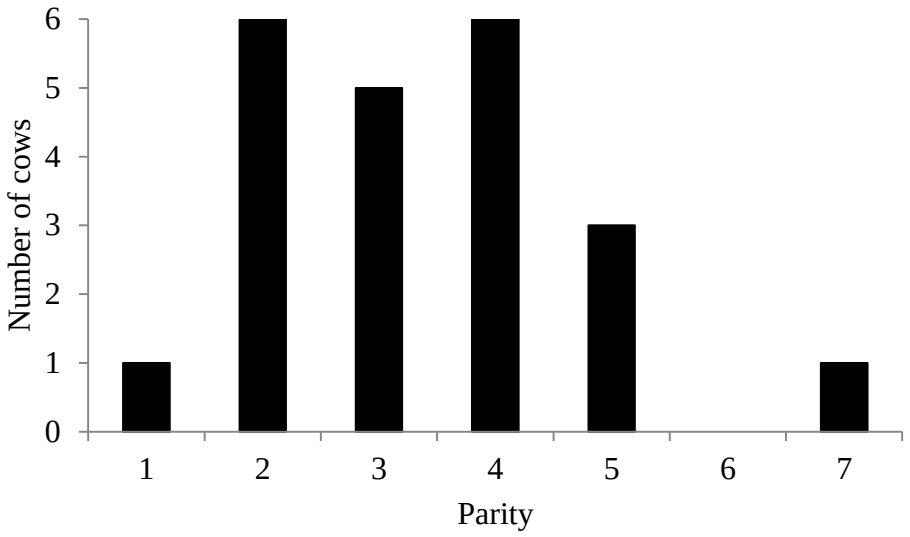

Figure 3.2: Number of cows with follicular cysts observed in each parity group.

3.3.1.3 Milk progesterone

Of the cysts diagnosed as follicular by the veterinarian 10/22 (45%) had high milk progesterone concentrations (≥3ng/ml). Thus the veterinary diagnosis 'disagreed' with the hormonal diagnosis, while the remaining 12 cows (55%) had low progesterone (<3ng/ml) thus the veterinary diagnosis 'agreed' with the hormonal diagnosis. Examples of milk progesterone profiles of cows whose veterinary diagnosis did or did not agree with progesterone concentrations are shown in Figure 3.3.

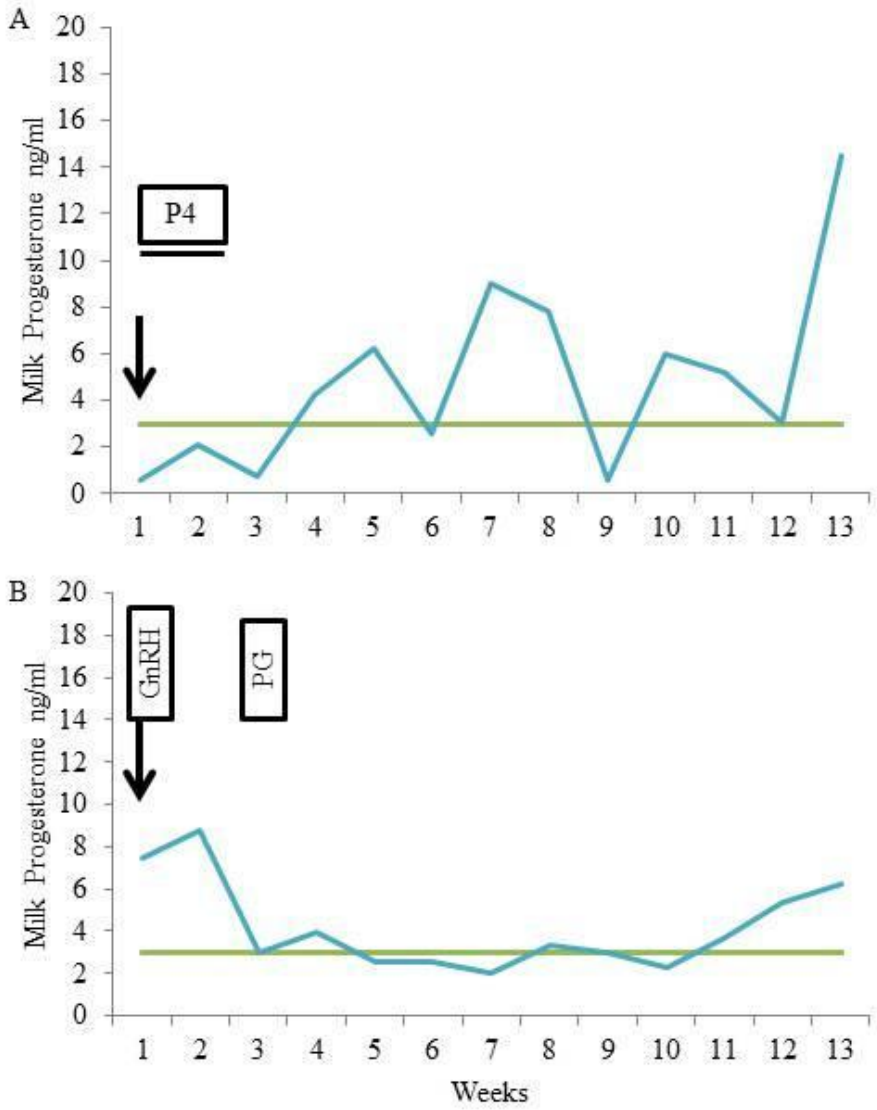

Figure 3.3: Milk progesterone profiles from two cows diagnosed by the veterinarian as having a follicular cyst. Progesterone concentrations above the green line are indicative of luteal activity (Darwash *et al.*, 1997). A) Progesterone concentrations confirmed, the cow had a follicular cyst at diagnosis (arrow), she was treated with progesterone (P4) for 14 days and resumed cyclicity (weeks 4-6). B) Milk progesterone concentrations indicated she did not have a follicular cyst at diagnosis; she was treated with GnRH at diagnosis and prostaglandin (PG) 14 days later but she did not resume cyclicity. The progesterone profile of this cow was similar during the first 3 weeks in all cows (n=10) whose veterinary diagnosis of a follicular cyst disagreed with the hormonal diagnosis.

3.3.1.4 Treatment outcome

Pregnancy was achieved by the end of the 13 week observation period post-treatment in 12/22 (53%) cows (Table 3.1). Conversely, 10/22 (40%) of cows failed to achieve pregnancy in the same time frame. Of these 10 cows, 4 resumed ovarian cyclic activity (determined by progesterone concentrations over three weeks measured as two high followed by one low (Bajema *et al.,* 1994)) while 6 cows developed a new ovarian cyst that required further treatment. Some cows (3/22) also presented clinical signs of an intrauterine infection after cyst diagnosis and were treated with a uterine wash-out, 500mg of Metricure (introduced into the lumen of the uterus) (MSD Animal Health, Milton Keynes, UK) and an injection (5ml, im) of Receptal (0.004mg/ml, MSD Animal Health, Milton Keynes, UK). One of these cows developed a further cyst whilst the other two failed to resume cyclicity or achieve pregnancy. The 13 week observation period was chosen to maximise the opportunity for pregnancy and/or resumption of cyclicity to be observed and had the observation period been shorter neither may have been detected.

Table 3.1: Contingency table displaying the treatment outcome within the 13 week observation period for cows diagnosed with a follicular cyst, and the agreement between the different diagnosis methods. No significant associations were found between agreement/disagreement of diagnoses and outcome of the treatments (Fishers Exact test).

PD+ = pregnancy successfully detected. [1]Veterinary diagnosis of a follicular cyst with low progesterone concentrations; [2]Veterinary diagnosis of a follicular cyst with high progesterone concentrations.

	PD +			
Diagnosis	Yes	No	TOTAL	P
Agree[1]	7	5	12	NS
Disagree[2]	5	5	10	
TOTAL	12	10	22	

3.3.2 Luteal cysts

3.3.2.1 Reported treatment regime

All cows with luteal cysts were treated at diagnosis, with an injection (2ml, im) of the prostaglandin $F_{2\alpha}$ analogue, cloprostenol (250µg/ml) (Estrumate, MSD Animal Health, Milton Keynes, UK) (n=8).

3.3.2.2 Parity and cysts

Of the cysts diagnosed as luteal, 6/8 (75%) were observed in cows between parity 2 and 4. There was only one cyst diagnosed in a cow of parity 1 and only one cyst diagnosed in cows of parity 5 or above (Figure 3.4). As these cows were not from one herd it is impossible to know the parity distribution of each herd from which these milk samples were collected from.

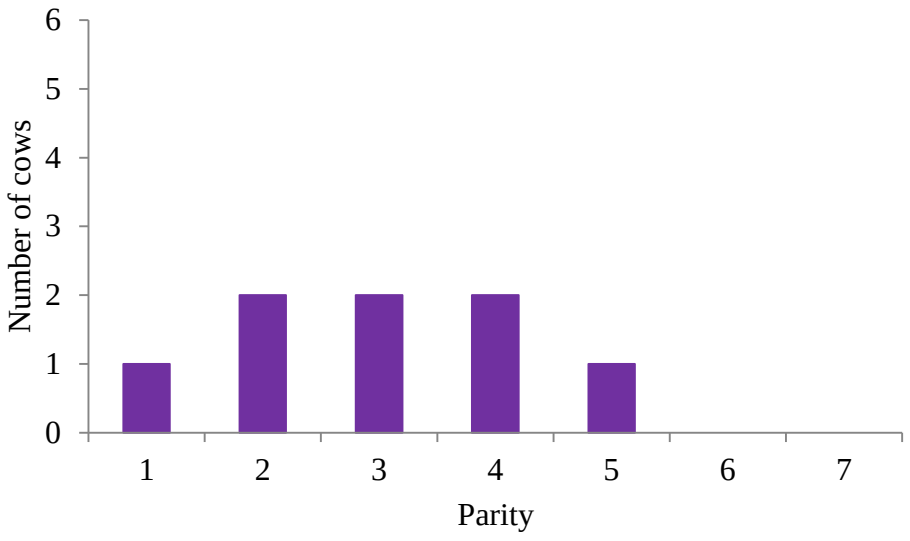

Figure 3.4: Number of cows with luteal cysts observed in each parity group.

3.3.2.3 Milk progesterone

Of the cysts diagnosed by the veterinarian as luteal, 3/8 (37.5%) had low milk progesterone concentrations (<3ng/ml) thus the veterinary diagnosis 'disagreed' with the hormone diagnosis. Conversely, 5/8 cows (62.5%) had high progesterone (≥3ng/ml) thus the veterinary diagnosis 'agreed' with the hormone diagnosis. An example of milk progesterone profiles of cows whose veterinary diagnosis did or did not agree with progesterone concentrations is shown in Figure 3.5.

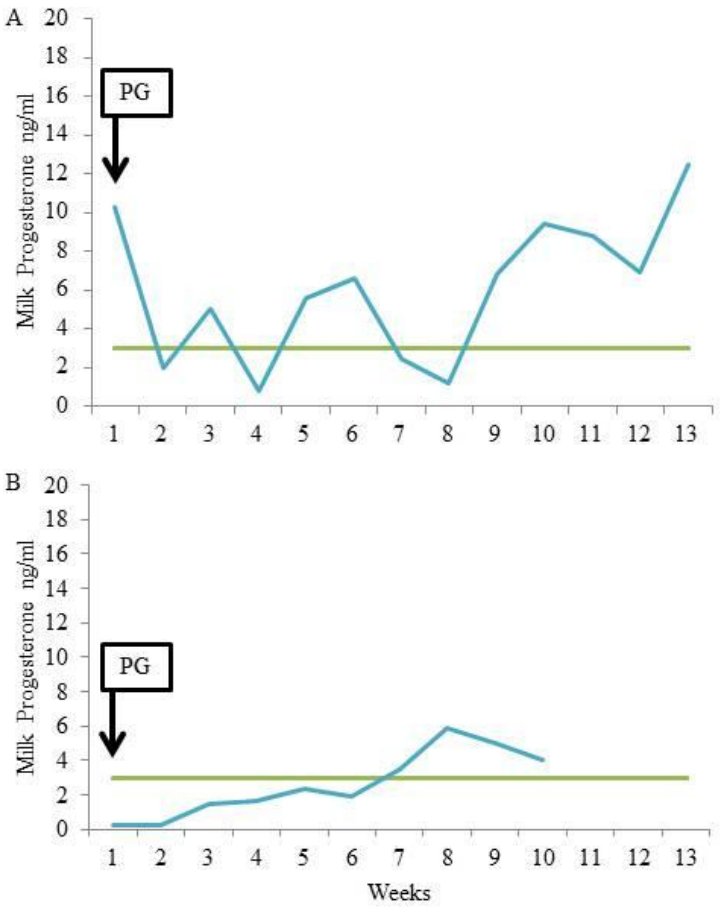

Figure 3.5: Milk progesterone profiles from two cows diagnosed by the veterinarian to have had a luteal cyst. Progesterone concentrations above the green line are indicative of luteal activity (Darwash *et al.*, 1997). A) Progesterone concentrations of this cow confirmed she had had a luteal cyst at diagnosis (arrow), she was treated with prostaglandin (PG) at diagnosis and resumed cyclicity (weeks 5-7). B) Milk progesterone concentrations indicated that the cystic structure did not have any luteal activity at diagnosis; she was also treated with prostaglandin at diagnosis but did not resume cyclicity. The progesterone profile of this cow was similar in all cows (n=3) whose veterinary diagnosis of a luteal cyst disagreed with the hormonal diagnosis.

3.3.2.4 Treatment outcome

Of cows diagnosed with a luteal cyst, 6/8 (75%) achieved pregnancy before the end of the 13 week observation period post-treatment (Table 3.2). Conversely, 2/8 (25%) cows failed to achieve pregnancy in the same time frame, but did successfully resume cyclic activity (based on previous reported criteria [Bajema *et al.*, 1994]) and no new cysts were observed.

Table 3.2: Contingency table displaying the treatment outcome within the 13 week observation period for cows diagnosed with a luteal cyst and the association the agreement between the different diagnosis methods. No significant associations were found between agreement/disagreement of diagnoses and outcome of the treatments (Fishers Exact test).

PD+ = pregnancy successfully detected. [1]Veterinary diagnosis of a luteal cyst with high progesterone concentrations; [2]Veterinary diagnosis of a luteal cyst with low progesterone concentrations.

	PD +			
Diagnosis	Yes	No	TOTAL	P
Agree[1]	3	2	5	NS
Disagree[2]	3	0	3	
TOTAL	6	2	8	

3.3.3 Diagnosis and choice of treatment

Of the 17 cows whose milk progesterone concentrations agreed with the diagnosis by veterinary assessment, all 17 received an appropriate choice of treatment and 10/17 (58.8%) became pregnant by the end of the observation period (Table 3.3). Of the 13 cows whose milk progesterone concentrations disagreed with the diagnosis by veterinary assessment, 10 (76.9%) retrospectively received an appropriate choice of treatment, of which 8 (61.5%) became pregnant by the end of the observation period (Table 3.4).

Table 3.3: Efficacy of treatment on the time until conception and alternate outcomes for cows whose cyst diagnosis by veterinary assessment agreed with milk progesterone concentrations at diagnosis.

Treatments: A) Progesterone intrauterine device for 14 days; PRID or CIDR from diagnosis. B) Injection (5ml, im) of the GnRH agonist buserelin at diagnosis. C) Injection (5ml, im) of the GnRH agonist buserelin at diagnosis, followed 14 days later by injection (2ml, im) of the prostaglandin $F_{2\alpha}$ analogue, cloprostenol. D) Injection (2ml, im) of the prostaglandin $F_{2\alpha}$ analogue, cloprostenol. FC = follicular cyst; LC = luteal cyst; PD+ = pregnancy confirmed; r = range.

Cyst type	Treatments	n	Number of cows PD+	Average number of weeks till conception	Other comments
FC	A	3	1	10	resumed cyclicity (n=2)
FC	B	3	2	12	new cyst (n=1)
FC	C	6	4	8.8 r: 8-11	resumed cyclicity (n=1) new cyst (n=1)
LC	D	5	3	10 r: 9-12	resumed cyclicity (n=2)
TOTAL		17	10		

Table 3.4: Efficacy of treatment on the time until conception and alternate outcomes for cows whose cyst diagnosis by veterinary assessment disagreed with milk progesterone concentrations at diagnosis.

Treatments: A) Injection (5ml, im) of the GnRH agonist buserelin acetate (0.004mg/ml) (Receptal, MSD Animal Health, Milton Keynes, UK) at diagnosis. B) Injection (5ml, im) of the GnRH agonist buserelin acetate (0.004mg/ml) (Receptal, MSD Animal Health, Milton Keynes, UK), at diagnosis, followed 14 days later by injection (2ml, im) of the prostaglandin $F_{2\alpha}$ analogue, cloprostenol (250µg/ml) (Estrumate, MSD Animal Health, Milton Keynes, UK). C) A double dose (10ml, im) of buserelin acetate (0.004mg/ml) (Receptal, MSD Animal Health, Milton Keynes, UK) at diagnosis followed 14 days later by injection (2ml, im) of the prostaglandin $F_{2\alpha}$ analogue, cloprostenol (250µg/ml) (Estrumate, MSD Animal Health, Milton Keynes, UK). D) Injection (2ml, im) of the prostaglandin $F_{2\alpha}$ analogue, cloprostenol (250µg/ml) (Estrumate, MSD Animal Health, Milton Keynes, UK). E) Injection (2ml, im) of the prostaglandin $F_{2\alpha}$ analogue, cloprostenol (250µg/ml) (Estrumate, MSD Animal Health, Milton Keynes, UK). FC =follicular cyst; LC = luteal cyst; PD+ = pregnancy confirmed; r = range.

Cyst type	Treatment	n	Number of cows PD+	Average number of weeks till conception	Other comments
FC	A	2	0	n/a	resumed cyclicity (n=1)
FC	B	4	4	10.25 r: 8-12	none
FC	C	1	0	n/a	new cyst (n=1)
FC	D	3	1	10	new cyst (n=1)
LC	E	3	3	9.7 r: 7-12	none
TOTAL		13	8		

In the group of cows whose veterinary diagnosis agreed with milk progesterone concentrations, of those that did not get pregnant (n=7) 2 cows developed a new cyst, and 5 cows successfully resumed cyclicity. Similarly for those cows whose veterinary diagnosis did not agree with milk progesterone concentrations, of those cows that did not get pregnant (n=5) 1 cow successfully resumed cyclicity, 2 cows developed a new cyst and 2 cows did not resume cyclicity at all across the entire observation period. Furthermore, 3 cows that were diagnosed by the veterinary to have a follicular cyst were administered 2ml (im) of the prostaglandin $F_{2\alpha}$ analogue, cloprostenol (250µg/ml) (Estrumate, MSD Animal Health, Milton Keynes, UK) as treatment, as the hormonal diagnosis found these cyst to be progestagenic, cloporsotenol is likely to have been effective against the luteal tissue present, resulting in 1 of the cows becoming pregnant (Table 3.4). Time from treatment to successful conception for cows whose diagnosis agreed or disagreed with milk progesterone concentrations is illustrated in Figure 3.6.

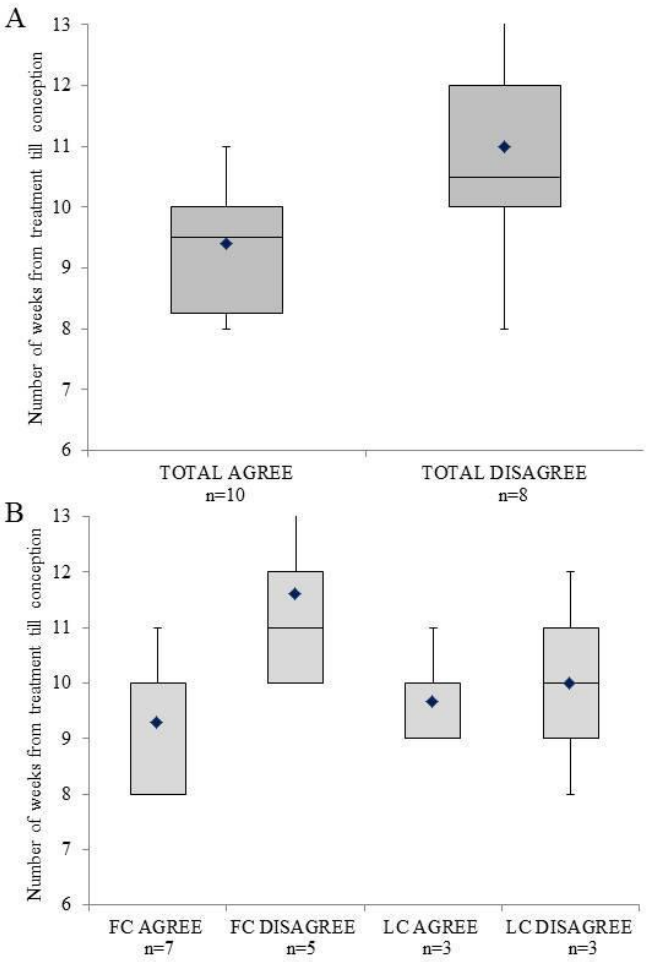

Figure 3.6: Number of weeks before conception was achieved after cyst diagnosis and treatment. The top and bottom of the box represents the upper and lower quartiles respectively, the line within the box represents the median value and the whiskers represents the minimum and maximum of the data set. The blue diamonds represent the mean value. A) For cows whose milk progesterone concentrations agreed with the veterinary assessment irrespective of cyst type, it took on average 9.4 weeks before conception was achieved. In cows whose milk progesterone concentrations disagreed with veterinary assessment irrespective of cyst type, it took on average 11 weeks before conception was achieved. B) Cows whose veterinary diagnosis of a follicular cyst (FC) agreed with milk progesterone concentrations took on average 9.3 weeks till successful conception (median = 10 weeks), or a luteal cyst (LC) took on average 9.7 weeks till successful conception (median = 9 weeks). Cows whose veterinary diagnosis of a follicular cyst disagreed with milk progesterone concentrations took on average 11.6 weeks till successful conception, or a luteal cyst took on average 10 weeks until successful conception. None of these differences were significant (ANOVA).

3.4 Discussion

This chapter has compared two diagnostic methods for the detection of follicular and luteal cysts in dairy cows. These comprised (1) routine veterinary diagnosis by rectal palpation and/or ultrasound and (2) progesterone measurements in milk. The effectiveness of these diagnoses used individually and combined has been assessed. When assessed individually, the success rates of both diagnostic methods were comparable (Figure 3.1, page 63). However, data presented in this chapter suggests that if a veterinary diagnosis is supported by a progesterone measurement, conception could be achieved up to two weeks earlier when compared to veterinary diagnosis alone.

Data presented in this chapter demonstrated that 43% of the cysts identified were misdiagnosed by veterinarian assessment based on milk progesterone concentrations of samples collected at diagnosis. Despite this, pregnancy rate within 13 weeks were similar whether the cyst diagnosis by veterinary assessment agreed or disagreed with milk progesterone concentrations (58.8% *vs.* 61.5%). However, cows whose milk progesterone concentrations agreed with cyst diagnosis by veterinary assessment, successfully conceived, on average, two weeks earlier than cows whose milk progesterone concentrations disagreed with veterinary cyst diagnosis, although this difference was not significant, most likely due to the large variation within cows whose hormonal and veterinary diagnoses disagreed.

These results concur with previous work in which Kendall, Mann & Flint (unpublished data) demonstrated that 38/109 (34.9%) ovarian cysts were incorrectly diagnosed, as determined by comparing veterinarian diagnosis with progesterone concentrations. The main differences between the two studies were that in Kendall *et al.,* study a fewer number of vets participated but with a greater number of farms

involved. Milk oestradiol concentrations were also measured to confirm cyst diagnosis but cows were not further monitored post diagnosis to track efficacy of treatment. Due to the high risk of contamination during the fat extraction process of the oestradiol assay and the expense involved, it was decided that determination of milk oestradiol was not logistically or financially possible for this thesis. The other important difference between the two trials was the criteria used at cyst diagnosis. During this trial structures fitting the classic definition of an ovarian cyst (page 39) were predominantly identified whereas Kendall *et al.*, included smaller persistent structures in their cyst classification.

Of the 30 cysts considered in this analysis, the veterinary assessment led to a diagnosis of 22 follicular and 8 luteal cysts. In contrast, the hormonal method led to a diagnosis of 16 follicular and 14 luteal cysts. Consequently, the success rates were comparable. Of all 30 cows diagnosed and treated, 3/30 (10%) received an inappropriate choice of treatment for their diagnosis (Table 3.4). This included the treatment of a follicular cyst with prostaglandin $F_{2\alpha}$, inappropriate as prostaglandin $F_{2\alpha}$ is routinely used to initiate luteolysis. However, the 3 cows diagnosed as having follicular cysts also had high progesterone concentrations and consequently the prostaglandin was an appropriate choice of treatment.

Although 54.5% and 75.0% of cows diagnosed by veterinary assessment with a follicular or luteal cyst (respectively) successfully achieved pregnancy, this was not before 8 weeks post diagnosis and treatment. As outlined in chapter 1 (page 41-42), treatment for both follicular and luteal cyst could be effective within 1-4 weeks of administration, dependent on cyst type, so although pregnancy was achieved eventually it may have been delayed by a failure at diagnosis to correctly differentiate between cyst types and thus administer the most appropriate treatment.

On further examination, these results suggest that conception, when hormonal diagnosis agreed with veterinary assessment, occurred on average two weeks earlier than when the diagnoses disagreed (Figure 3.6). Delayed conception can have substantial economic costs to the farmer, indeed research has shown that there is a reduction in milk yield by 2.4kg as well as a reduction in milk fat by 0.112kg with each additional day open (Louca & Legates, 1968). If these figures are applied to the results in this chapter then over the course of the average two additional weeks open each cow misdiagnosed would lose 33.6kg in milk yield and 1.57kg in milk fat. Furthermore, there is an increased cost of culling cows repeatedly failing to achieve pregnancy, together, this reduction in yield and increase in the cost of culling and replacing cows has been estimated to cost the farmer approximately 10% of their income per year (Dijkhuizen *et al.*, 1985). This does not include the additional cost of veterinary visits or chosen treatments. As 43% of cysts were found to be misdiagnosed this could add up to a serious loss to the farmer, and clearly prompts a fuller investigation of suspected ovarian cysts at diagnosis by their veterinarian.

There were no additional delays in time to conception whether the veterinary luteal cyst diagnosis agreed or not with milk progesterone concentrations. All luteal cyst diagnoses, irrespective of agree or disagree, were treated with one treatment protocol Injection (2ml, im) of the prostaglandin $F_{2\alpha}$ analogue, cloprostenol (250µg/ml) (Estrumate, MSD Animal Health, Milton Keynes, UK). Three of the cows diagnosed by veterinary assessment to have a luteal cyst were demonstrated to be non-progestagenic, so treatment with a prostaglandin analogue is unlikely to be effective. However, all three of these cows successfully achieved pregnancy, although it was on average 9.7 weeks after treatment. This length of time in between treatment and conception may have given them enough time to spontaneously

resolve any ovarian cysts and recover naturally before achieving pregnancy. A review of their individual progesterone profiles (not included in this thesis) demonstrated that treatment with the prostaglandin analogue had no effect on decreasing milk progesterone concentrations, conversely in two of these cows milk progesterone concentrations actually increased. This increase in progesterone may reflect the presence of a newly formed CL at the time that PG was administered i.e., non-responsive at that stage.

Kendall, Mann & Flint (unpublished data), combined with this chapter's results reiterate the importance of a hormonal diagnosis when differentiating ovarian cyst type. Currently, there are three companies that supply "on-farm" progesterone kits that can be used to determine progesterone concentrations in either milk or serum samples. These companies are Ridgeway Science (Gloucestershire, UK), Target (NJ, USA) and Accufirm (ME, USA). The aim of these tests is not to give an accurate concentration of progesterone, but instead to give an indication of 'high' or 'low' progesterone concentrations. The use of these progesterone kits to differentiate between cyst type has been shown to increase pregnancy rates ($P<0.08$) by day 210 postpartum, as well as significantly decreasing the number of days open ($P<0.05$) (Bajema *et al.*, 1994). This amounts to a cost saving of $70.42 (US) per cow (correct in 1994, Bajema *et al.*, 1994). Furthermore, use of progesterone kits on farm can extend to; 1) identifying errors in the detection of oestrus, 2) predicting time of oestrus, 3) assisting pregnancy diagnosis, and 4) evaluating response to endocrine therapy (Nebel, 1988), thus can be considered a valuable asset in managing dairy cow fertility.

Of the two cows that developed an intrauterine infection, one was diagnosed two weeks post treatment for this infection, with a follicular cyst. Intrauterine

infections have been demonstrated to impair ovarian function post-partum (Mateus *et al.,* 2002) and increase the risk of developing an ovarian cyst (Tsousis *et al.,* 2009). It has been hypothesised that endotoxins present in the uterus are released into the bloodstream, affecting cortisol concentrations and impairing an LH surge (Bosu & Peter, 1987). The absence of an LH surge could lead to persistence of a large oestrogenic follicle (Figure 1.8, page 45), and increased cortisol levels could result in an decrease of baseline LH concentrations and a decrease in LH pulse frequency which could also potentially lead to the development of a large an-ovulatory steroidogenic ovarian cyst.

Furthermore, these results have also identified a problem with current treatment protocols, even when a correct diagnosis was made by the veterinarian (confirmed by milk progesterone concentrations) and an accurate treatment protocol was chosen, cows did not become pregnant before 8 weeks post-treatment. This may indicate that current therapies available for the treatment of ovarian cysts are no longer as effective as was once thought, and suggests more research is needed in order to better manage and treat the problem of ovarian cysts.

3.5 Limitations

Whilst undertaking the progesterone assays, higher than desirable % CV values for both upper and lower QCs were recorded so samples were repeated to ensure validity of the results. It is likely that this variation has resulted due to the large number of assays run to obtain the results for this chapter. Furthermore, hormone determination in milk is disadvantageous when compared to hormone concentrations determined in serum. Serum samples remain stable for longer than milk samples and results are likely to be more consistent (Stokol & Nydam, 2005). Considering that sample

collection was performed by farmers, withdrawal of blood on a weekly basis would not have been a feasible option for this study, however there would be the possibility of a blood sample taken by the veterinarian at cyst diagnosis for progesterone determination to be combined with weekly milk collection.

The results from this study indicate that ovarian cysts are increasingly prevalent in multiparous *vs.* primiparous cows. However, primiparous cows were not sufficiently represented in this study (n=2) so conclusions cannot be drawn regarding frequency of cysts and increasing parity. The same can be said for cows above parity 5 (n=2), as we do not know the herd parity profiles.

The number of cows recruited onto this study was low; in order to increase the impact of these findings the study would need to be repeated with the inclusion of a greater number of cows. This would facilitate the development of survival curves that would demonstrate the effect of a misdiagnosis on long-term profitability of a dairy herd.

3.6 Conclusion

In conclusion, differentiating between ovarian cyst types is still an issue for practicing veterinarians. As a result, failure of chosen treatments is delaying resumption of cyclicity and achieving pregnancy. Progesterone is invaluable in the accurate differentiation of ovarian cyst types that needs re-introducing as a common diagnostic tool to be used **alongside** transrectal ultrasonography and rectal palpation.