ABSTRACT

Ovarian cysts are a cause of reproductive failure and economic loss in postpartum dairy cows. Using a unique combination of research to approach this problem, this thesis aimed to better understand mechanisms of ovarian cyst formation.

The use of progesterone as a tool in cyst diagnosis was initially examined. Results demonstrated that 13/30 (43%) cows had progesterone profiles that disagreed with veterinarian diagnosis. Furthermore treatment in 21/30 (70%) cows was ineffective within 4 weeks of administration, and no pregnancy was established earlier than 8 weeks post treatment in all cows. When veterinarian and hormonal diagnosis agreed pregnancy was achieved, on average, two weeks earlier than when they disagreed.

Effects of cow management, specifically the NEB experienced during late gestation and early lactation were investigated to determine whether these increased requirements resulted in the development of ovarian cysts. Results demonstrated that from early lactation all 85 cows were in a state of NEB. Ovarian cysts were confirmed in 31/79 cows, and these cows had significantly higher or lower peripheral concentrations of some metabolites, *vs.* no-cyst cows.

Long term down-regulation with a GnRH agonist, followed by a period of observation to monitor the recovery of reproductive function, was conducted for evaluation as a potential model for ovarian cyst formation. Results indicated that 6/12 cows exhibited an LH surge within 104 hours of luteal regression while 6 animals did not (P<0.001). FSH concentrations in 6/12 cows showed divergence comparable with LH surges. 8/12 had at least 1 follicle >8mm and 5/12 had at least 1 follicle >20 mm. Follicle appearance was heterogeneous, with 63% of follicles showing some degree of luteinisation. Positive immunostaining for steroidogenic enzymes was detected in 12.5% of follicles.

In conclusion, these results have important clinical significance in improving the diagnosis and management of ovarian cysts in dairy cows.

PUBLICATIONS ARISING FROM THIS THESIS

JACKSON, R.A., WILLS, J.R., KENDALL, N.R., GREEN, M.J., MURRAY, R.D., DOBSON, H. 2011. Energy metabolites in pre- and postpartum dairy cattle as predictors of reproductive disorders. *Veterinary Record.* 168: 562-567

ACKNOWLEDGEMENTS

I would like to start by thanking my supervisors Dr Nigel Kendall, Dr Richard Lea, Dr George Mann and Prof Bruce Campbell. Thanks also go to Dr Bob Robinson and Prof Clare Wathes for giving me the opportunity to complete this project. Thanks go to Mrs Catherine Pincott-Allen for, not only, the countless hours we spent getting those RIAs right despite sweltering temperatures in the lab, but also for your friendship and support. Thanks to Mrs Morag Hunter and to Juan Hernandez for your technical assistance, time, friendship and guidance in the lab. I would also like to thank the vets; Mark Burnell, Julian Allen and Rachel Hayton, particularly Mark, for their support in recruiting farmers onto my trials. My PhD experience would not have been the same without one whole year of early morning farm visits to Joe, Paul, Arthur and Michael who, despite the trial over running by 6 months, never complained once. I would like to thank Dawn from JF Cobb and Sons, for her diligent collection and labelling of hundreds of milk samples and her continued support and belief in my project, as well as Dawn, thanks go to all the other farmers that also participated in this study to make it a success. I would also like to thank Jaime Hughes for her support and friendship from the very beginning of my project, you never turned me away when I had a question, I enjoyed our stolen chats in the corridor and I will never stop laughing at some of things you said to me! Furthermore, thanks to Leanne Williams and Sarah Potterton for your support during these difficult last few months.

Most importantly, I would like to thank my Mum and Dad, and my brothers James and George, for their support during the last 5 years, at times it has been very difficult but you all kept me motivated and your words of positivity and encouragement kept me going. I would also like to thank Rella, not only for her ceaseless ability to make me laugh, but also for her unconditional love and support all the way through this project, thank you for believing in me. Thanks also go to Hamish for supporting me, in more ways than one, over the last 18 months, you have been instrumental in the completion of this thesis and I am very grateful, so thank you.

Last but not least I would like to thank the IDTC and Nottingham University for providing the funding that gave me the opportunity to complete this project.

TABLE OF CONTENTS

	PAGE
Chapter 1 Literature Review	1
1.1 Ovarian Function	2
1.2 Control of ovarian dynamics	3
1.3 Folliculogenesis	7
1.3.1 Follicle classification	7
1.3.2 Overall pattern of growth	9
1.3.3 Pre-antral follicle growth and regulation	10
1.3.4 Antral follicle growth	16
1.3.5 Recruitment of follicles	24
1.3.6 Selection for the dominant follicle	24
1.3.7 Follicular dominance	28
1.4 Endocrine and ovarian events prior to ovulation	31
1.5 Luteinisation and formation of a corpus luteum	32
1.6 Follicular atresia	34
1.6.1 Atresia in large antral follicles	35
1.7 Bovine ovarian cysts	39
1.7.1 Cyst diagnosis	40
1.7.2 Treatment of ovarian cysts	41
1.7.3 Aetiology of ovarian cysts	43
1.7.4 Transition period, and reproductive pathologies associated with the postpartum period in lactating dairy cows	46

1.7.5 Stress induced ovarian cysts	46
1.7.5.1 Metabolic stress	47
1.7.5.2 Heat stress	49
1.7.5.3 Genetic stress	51
1.8 Hypotheses	53
Chapter 2 Materials and Methods	54
2.1 Blood collection	54
2.2 Plasma progesterone enzyme-linked immuno-sorbent assay	54
2.3 Oestradiol radioimmunoassay	55
Chapter 3 Diagnostic Study	59
3.1 Materials and methods	59
3.1.1 On-farm protocol	59
3.1.2 Milk collection	60
3.1.3 Milk progesterone enzyme-linked immuno-sorbent assay	60
3.2 Data analysis	61
3.3 Results	63
3.3.1 Follicular cysts	64
3.3.1 1 Reported treatment regimes	64
3.3.1.2 Parity and cysts	64
3.3.1.3 Milk progesterone	65
3.3.1.4 Treatment outcome	67
3.3.2 Luteal cysts	68

3.3.2.1 Reported treatment regime	68
3.3.2.2 Parity and cysts	68
3.3.2.3 Milk progesterone	69
3.3.2.4 Treatment outcome	71
3.3.3 Diagnosis and choice of treatment	71
3.4 Discussion	76
3.5 Limitations	80
3.6 Conclusion	81
Chapter 4 Management Study	82
4.1 Serum parameters used in energy profiling	83
4.2 Body condition scoring	85
4.3 Materials and methods	85
4.3.1 Metabolite analyses	87
4.3.2 Progesterone concentration	90
4.3.3 Statistical analysis	90
4.4 Results	91
4.4.1 Results from cows bearing single offspring	91
4.4.2 Energy parameters (singletons)	91
4.4.3 Liver parameters (singletons)	98
4.4.4 Progesterone profiles (singletons)	108
4.4.5 Calving to conception intervals and 305d milk yield data for	110
cows bearing single offspring	
4.4.6 Early versus late ovarian pathology	111

4.4.7 Effect of cows bearing twin offspring on metabolic	112
parameters	
4.4.7.1 Energy parameters (twins)	112
4.4.7.2 Liver parameters (twins)	114
4.4.10 Calving to conception intervals and 305d milk yield	118
data for cows bearing twin offspring	
4.5 Discussion	119
4.5.1 Cyst vs. no cyst comparisons in cows bearing single	119
offspring	
4.5.2 Twin vs. single comparisons	122
4.6 Strengths and limitations	123
4.7 Conclusion	125
Chapter 5 Model for the Study of Ovarian Cysts	126
5.1 Materials and methods	126
5.1.1 Hyper-stimulation protocol	126
5.1.2 Post-buserelin model for ovarian cysts	128
5.1.3 Laboratory protocols	131
5.1.3.1 Follicle dissection	131
5.1.3.2 Pituitary dissection	131
5.1.3.3 Tissue processing	132
5.1.3.4 Measurement of hormones	133
5.1.3.4.1 Radioimmunoassay	133
5.1.3.4.2 Enzyme linked immuno-sorbent assays (ELISAs)	136
5.1.3.5 Immunohistochemistry	138

5.1.4 Statistical analysis	141
5.2 Results	143
5.2.1 Dynamics of ovarian follicle growth and development	143
5.2.2 Peripheral hormone concentrations	144
5.2.3 Follicle size and intra-follicular steroid hormone concentrations	146
5.2.4 Immunohistochemistry	150
5.2.5 LH pulse data	152
5.3 Discussion	156
5.4 Limitations	158
5.5 Conclusion	160
Chapter 6 General Discussion	161
6.1 Clinical relevance of these findings	163
6.2 Limitations of this work	170
6.3 Final Conclusions	172
BIBLIOGRAPHY	173

LIST OF FIGURES

	PAGE
Figure 1.1: Layers of the ovary.	2
Figure 1.2: Steroidogenic profiles during the bovine oestrous cycle.	4
Figure 1.3: A schematic representation of positive and negative feedback	6
when there is a dominant follicle or a corpus luteum as the dominant	
structure on the ovary.	
Figure 1.4: A schematic representation of follicle development from	7
primordial follicles through to post ovulation and corpus luteum formation.	
Figure 1.5: A summary of the interactions between various growth factors	13
regulating pre-antral follicle growth.	
Figure 1.6: A summary of steroid hormone synthesis in the granulosa and	22
thecal layers.	
Figure 1.7: LH and FSH interactions through their receptors with a	27
granulosa cell post-deviation and their effect in up-regulating LHr to aid	
development of the aromatase enzyme complex.	
Figure 1.8: A summary of expected follicle growth and interference points	45
and their possible effects leading to the disruption of normal follicle	
development.	
Figure 1.9: A summary of the effects of heat stress in the lactating dairy	49
cow.	
Figure 3.1: Number of follicular and luteal cysts identified by veterinary	63
assessment or hormonal diagnosis.	
Figure 3.2: Number of follicular cysts observed in each parity group.	65
Figure 3.3: Milk progesterone profiles from two cows diagnosed by the	66
veterinarian to have had a follicular cyst.	
Figure 3.4: Number of luteal cysts observed in each parity group.	68
Figure 3.5: Milk progesterone profiles from two cows diagnosed by the	70
veterinarian to have had a luteal cyst.	
Figure 3.6: Number of weeks before conception was achieved after cyst	75
diagnosis and treatment.	

Figure 4.1: Non-esterified fatty acid concentrations in pre- and post-	94
calving cows over a 15 week period.	
Figure 4.2: β-hydroxy butyrate concentrations in pre- and post-calving	95
cows over a 15 week period.	
Figure 4.3: Urea concentrations in pre- and post-calving cows over a 15	96
week period.	
Figure 4.4: Body condition score in pre- and post-calving cows over a 15	97
week period.	
Figure 4.5: Triglyceride concentrations in pre- and post-calving cows over	99
a 15 week period.	
Figure 4.6: Cholesterol concentrations in pre- and post-calving cows over a	100
15 week period.	
Figure 4.7: γ-Glutamyl transpeptidase activities in pre- and post-calving	102
cows over a 15 week period.	
Figure 4.8: Aspartate transaminase activities in pre- and post-calving cows	103
over a 15 week period.	
Figure 4.9: Total protein concentrations in pre- and post-calving cows over	105
a 15 week period.	
Figure 4.10: Albumin concentrations in pre- and post-calving cows over a	106
15 week period.	
Figure 4.11: Globulin concentrations in pre- and post-calving cows over a	107
15 week period.	
Figure 4.12: Mean progesterone concentrations in pre- and post-calving	108
cows over a 15 week period.	
Figure 4.13: Progesterone profiles of cows that did and did not develop an	109
ovarian cyst.	
Figure 4.14: Concentrations of NEFA, BHB, urea, as well as body	113
condition score, in twin bearing vs. single bearing cows.	
Figure 4.15: Concentrations of triglycerides and cholesterol in twin bearing	115
vs. single bearing cows.	
<u>Figure 4.16</u> : Total activity of γ -Glutamyl transpeptidase and aspartate	116
transaminase in plasma of twin vs. single bearing cows.	

Figure 4.17: Concentrations of total protein, albumin, and globulin in twin	117
bearing vs. single bearing cows.	
Figure 5.1: Summary view of the hyper-stimulation model.	128
Figure 5.2: Peripheral concentrations of luteinising hormone in heifers that	144
did and did not exhibit an LH surge across the 120 hours following	
administration of the prostaglandin analogue, cloprostenol.	
Figure 5.3: Peripheral concentrations of follicle stimulating hormone in	145
heifers that did and did not exhibit an LH surge across 120 hours following	
administrations of the prostaglandin analogue, cloprostenol.	
Figure 5.4: Comparison of the range of follicle size (A) and intra-follicular	148
steroid hormone concentrations, oestradiol (B), progesterone (C) and	
androstenedione (D), between the no-surge and surge heifers.	
Figure 5.5: Immunolocalisation of the expression of LH and FSH in	150
pituitary gland.	
Figure 5.6: Immunolocalisation of the expression of aromatase and	151
P450c17 in follicular tissue.	
Figure 5.7: Pulsatile release of luteinising hormone during the four hour	
pulse bleed in heifers that did not exhibit an LH surge.	
Figure 5.8: Pulsatile release of luteinising hormone during the four hour	153
pulse bleed in heifers that did exhibit an LH surge.	
Figure 5.9: LH pulse characteristics in heifers with and without an LH	154
surge.	

LIST OF TABLES

	PAGE
Table 1.1: A summary of key ligands and the location of their receptors.	3
Table 1.2: Classification and characterisation of bovine follicles.	8
Table 1.3: A summary of the role played by key gonadotropins, hormones and	12
growth factors during pre-antral follicle development.	
Table 1.4: A summary of the role played by key gonadotropins, hormones and	17
growth factors during type 5 follicle development.	
Table 1.5: A summary of the role played by key gonadotropins, hormones and	23
growth factors during type 6 follicle development.	
Table 1.6: A summary of the role played by key gonadotropins, hormones and	31
growth factors during type 7 follicle development.	
Table 1.7: Summary of gonadotropins, hormones and growth factors	38
associated with atresia of pre-antral, antral and pre-ovulatory follicles vs.	
healthy follicles of the same size.	
Table 1.8: A summary of fluctuating concentrations of steroid hormones and	51
gonadotropins, during follicular dominance (or lack of) throughout the year.	
Table 2.1: Quantity of reagents, radioactive tracer and antibodies in non-	58
specific binding, total count, total bound, standard curve, quality controls and	
samples for the oestradiol MAIA RIA.	
Table 3.1: Contingency table displaying diagnosis agreement and treatment	67
outcome for cows diagnosed with a follicular cyst within the 13 week	
observation period.	
Table 3.2: Contingency table displaying diagnosis agreement and treatment	71
outcome for cows diagnosed with a luteal cyst within the 13 week observation	
period.	
Table 3.3: Efficacy of treatment on the time until conception and alternate	72
outcomes for cows whose cyst diagnosis by veterinary assessment agreed with	
milk progesterone concentrations at diagnosis.	
Table 3.4: Efficacy of treatment on the time until conception and alternate	73
outcomes for cows whose cyst diagnosis by veterinary assessment disagreed	
with milk progesterone concentrations at diagnosis.	

Table 4.1:Individual kit codes for each metabolite plus inter-assay %88coefficient of variation values for the 3 different lots of bovine sera analytelevel II QC used.

Table 4.2: Expected normal values of blood metabolites and implications of89elevated/subnormal concentrations postpartum for Holstein-Friesian dairycows during the transition period.

Table 4.3:Summary of parameters that significantly differed from the control109no-cyst group at each time point for cows bearing single offspring, sorted bycyst type.

Table 4.4:Summary of calving to conception intervals in each group between110the conception following cyst diagnosis (or not) and subsequent calving.

Table 4.5:Summary of 305d milk yield data for cows in each group prior to110starting the trial.

Table 4.5:Summary of parameters in cows bearing twins that significantly118differed from cows bearing singles at each time point.

Table 4.6:Summary of calving to conception intervals for twins and singles118between the conception following cyst diagnosis (or not) and subsequentcalving.

Table 4.7:Summary of 305d milk yield data in twins and singles prior to119starting the trial.

Table 5.1: A summary of the treatment protocol for groups A and B for the130post Buserelin experimental protocol.

<u>Table 5.2:</u> Quantity of reagents, radioactive tracer, antibodies and wash solution in non-specific binding, total count, total bound, standard curve, quality controls and samples for luteinising hormone, follicle stimulating hormone, androstenedione and, oestradiol radioimmunoassays.

Table 5.3:Summary of differences in methodology between the progesterone138and oestradiol enzyme linked immuno-sorbent assays.

Table 5.4: Number of follicles present at dissection, following euthanasia at143120 hours post prostaglandin.

Table 5.5:Steroid hormone concentrations in the follicular fluid of follicles147 \geq 8mm diameter in heifers that did not exhibit an LH surge.

Table 5.6:Steroid hormone concentrations in the follicular fluid of follicles147 \geq 8mm diameter in heifers that exhibited an LH surge.

Table 5.7:Summary of average follicle size, follicular fluid hormone149concentrations and oestradiol:progesterone ratios, with standard error of themean between the surge and no-surge heifers on average and within thelargest follicles for each group.

Table 5.8: LH pulse characteristics in heifers with and without an LH surge. 155

LIST OF ABBREVIATIONS

3β-HSD3β-hydroxy-steroid dehydrogenaseA4androstenedioneABavitin-biotinACadenylate cyclaseACTHadrenocorticotropic hormoneANOVAanalysis of varianceAPanterior pituitaryASTaspartate transaminaseBCSbody condition scorebFGFbasic fibroblast growth factorBHBbeta-hydroxy butyrateBMPbone morphogenetic proteinBSAbovine serum albumincAMPcyclic adenosine monophosphateCCIcalving to conception intervalCLcorpus luteum/corpora luteaCOCcumulus oophorus complexCPMcounts per minuteCVcoefficient of varianceddayDAB3,3'-diaminobenzidineDFdominant follicleDMIdry matter intakeE2oestradiolEDTAethylenediaminetetraacetic acidEGFepidermal growth factorELISAenzyme-linked immuno-sorbent assayFCfollicular cystFMEfermentable metabolisable energy
ACadenylate cyclaseACTHadrenocorticotropic hormoneANOVAanalysis of varianceAPanterior pituitaryASTaspartate transaminaseBCSbody condition scorebFGFbasic fibroblast growth factorBHBbeta-hydroxy butyrateBMPbone morphogenetic proteinBSAbovine serum albumincAMPcyclic adenosine monophosphateCCIcalving to conception intervalCLcorpus luteum/corpora luteaCOCcumulus oophorus complexCPMcounts per minuteCVcoefficient of varianceddayDAB3,3'-diaminobenzidineDFdominant follicleDMIdry matter intakeE2oestradiolEDTAethylenediaminetetraacetic acidEGFepidermal growth factorELISAenzyme-linked immuno-sorbent assayFCfollicular cyst
ACTHadrenocorticotropic hormoneANOVAanalysis of varianceAPanterior pituitaryASTaspartate transaminaseBCSbody condition scorebFGFbasic fibroblast growth factorBHBbeta-hydroxy butyrateBMPbone morphogenetic proteinBSAbovine serum albumincAMPcyclic adenosine monophosphateCCIcalving to conception intervalCLcorpus luteum/corpora luteaCOCcumulus oophorus complexCPMcoefficient of varianceddayDAB3,3'-diaminobenzidineDFdominant follicleDMIdry matter intakeE2oestradiolEDTAenzyme-linked immuno-sorbent assayFCfollicular cyst
ACTHadrenocorticotropic hormoneANOVAanalysis of varianceAPanterior pituitaryASTaspartate transaminaseBCSbody condition scorebFGFbasic fibroblast growth factorBHBbeta-hydroxy butyrateBMPbone morphogenetic proteinBSAbovine serum albumincAMPcyclic adenosine monophosphateCCIcalving to conception intervalCLcorpus luteum/corpora luteaCOCcumulus oophorus complexCPMcoefficient of varianceddayDAB3,3'-diaminobenzidineDFdominant follicleDMIdry matter intakeE2oestradiolEDTAethylenediaminetetraacetic acidEGFepidermal growth factorELISAenzyme-linked immuno-sorbent assayFCfollicular cyst
ANOVAanalysis of varianceAPanterior pituitaryASTaspartate transaminaseBCSbody condition scorebFGFbasic fibroblast growth factorBHBbeta-hydroxy butyrateBMPbone morphogenetic proteinBSAbovine serum albumincAMPcyclic adenosine monophosphateCCIcalving to conception intervalCLcorpus luteum/corpora luteaCOCcumulus oophorus complexCPMcoefficient of varianceddayDAB3,3'-diaminobenzidineDFdominant follicleDMIdry matter intakeE2oestradiolEGFepidermal growth factorELISAenzyme-linked immuno-sorbent assayFCfollicular cyst
APanterior pituitaryASTaspartate transaminaseBCSbody condition scorebFGFbasic fibroblast growth factorBHBbeta-hydroxy butyrateBMPbone morphogenetic proteinBSAbovine serum albumincAMPcyclic adenosine monophosphateCCIcalving to conception intervalCLcorpus luteum/corpora luteaCOCcumulus oophorus complexCPMcoefficient of varianceddayDAB3,3'-diaminobenzidineDFdominant follicleDMIdry matter intakeE2oestradiolEDTAethylenediaminetetraacetic acidEGFepidermal growth factorELISAenzyme-linked immuno-sorbent assayFCfollicular cyst
ASTaspartate transaminaseBCSbody condition scorebFGFbasic fibroblast growth factorBHBbeta-hydroxy butyrateBMPbone morphogenetic proteinBSAbovine serum albumincAMPcyclic adenosine monophosphateCCIcalving to conception intervalCLcorpus luteum/corpora luteaCOCcumulus oophorus complexCPMcounts per minuteCVcoefficient of varianceddayDAB3,3'-diaminobenzidineDFdominant follicleDMIdry matter intakeE2oestradiolEDTAethylenediaminetetraacetic acidEGFepidermal growth factorELISAenzyme-linked immuno-sorbent assayFCfollicular cyst
bFGFbasic fibroblast growth factorBHBbeta-hydroxy butyrateBMPbone morphogenetic proteinBSAbovine serum albumincAMPcyclic adenosine monophosphateCCIcalving to conception intervalCLcorpus luteum/corpora luteaCOCcumulus oophorus complexCPMcounts per minuteCVcoefficient of varianceddayDAB3,3'-diaminobenzidineDFdominant follicleDMIdry matter intakeE2oestradiolEDTAethylenediaminetetraacetic acidEGFepidermal growth factorELISAenzyme-linked immuno-sorbent assayFCfollicular cyst
bFGFbasic fibroblast growth factorBHBbeta-hydroxy butyrateBMPbone morphogenetic proteinBSAbovine serum albumincAMPcyclic adenosine monophosphateCCIcalving to conception intervalCLcorpus luteum/corpora luteaCOCcumulus oophorus complexCPMcounts per minuteCVcoefficient of varianceddayDAB3,3'-diaminobenzidineDFdominant follicleDMIdry matter intakeE2oestradiolEDTAethylenediaminetetraacetic acidEGFepidermal growth factorELISAenzyme-linked immuno-sorbent assayFCfollicular cyst
BMPbone morphogenetic proteinBSAbovine serum albumincAMPcyclic adenosine monophosphateCCIcalving to conception intervalCLcorpus luteum/corpora luteaCOCcumulus oophorus complexCPMcounts per minuteCVcoefficient of varianceddayDAB3,3'-diaminobenzidineDFdominant follicleDMIdry matter intakeE2oestradiolEDTAethylenediaminetetraacetic acidEGFepidermal growth factorELISAenzyme-linked immuno-sorbent assayFCfollicular cyst
BSAbovine serum albumincAMPcyclic adenosine monophosphateCCIcalving to conception intervalCLcorpus luteum/corpora luteaCOCcumulus oophorus complexCPMcounts per minuteCVcoefficient of varianceddayDAB3,3'-diaminobenzidineDFdominant follicleDMIdry matter intakeE2oestradiolEDTAethylenediaminetetraacetic acidEGFepidermal growth factorELISAenzyme-linked immuno-sorbent assayFCfollicular cyst
cAMPcyclic adenosine monophosphateCCIcalving to conception intervalCLcorpus luteum/corpora luteaCOCcumulus oophorus complexCPMcounts per minuteCVcoefficient of varianceddayDAB3,3'-diaminobenzidineDFdominant follicleDMIdry matter intakeE2oestradiolEDTAethylenediaminetetraacetic acidEGFepidermal growth factorELISAenzyme-linked immuno-sorbent assayFCfollicular cyst
CCIcalving to conception intervalCLcorpus luteum/corpora luteaCOCcumulus oophorus complexCPMcounts per minuteCVcoefficient of varianceddayDAB3,3'-diaminobenzidineDFdominant follicleDMIdry matter intakeE2oestradiolEDTAethylenediaminetetraacetic acidEGFepidermal growth factorELISAenzyme-linked immuno-sorbent assayFCfollicular cyst
CLcorpus luteum/corpora luteaCOCcumulus oophorus complexCPMcounts per minuteCVcoefficient of varianceddayDAB3,3'-diaminobenzidineDFdominant follicleDMIdry matter intakeE2oestradiolEDTAethylenediaminetetraacetic acidEGFepidermal growth factorELISAenzyme-linked immuno-sorbent assayFCfollicular cyst
COCcumulus oophorus complexCPMcounts per minuteCVcoefficient of varianceddayDAB3,3'-diaminobenzidineDFdominant follicleDMIdry matter intakeE2oestradiolEDTAethylenediaminetetraacetic acidEGFepidermal growth factorELISAenzyme-linked immuno-sorbent assayFCfollicular cyst
CPMcounts per minuteCVcoefficient of varianceddayDAB3,3'-diaminobenzidineDFdominant follicleDMIdry matter intakeE2oestradiolEDTAethylenediaminetetraacetic acidEGFepidermal growth factorELISAenzyme-linked immuno-sorbent assayFCfollicular cyst
CVcoefficient of varianceddayDAB3,3'-diaminobenzidineDFdominant follicleDMIdry matter intakeE2oestradiolEDTAethylenediaminetetraacetic acidEGFepidermal growth factorELISAenzyme-linked immuno-sorbent assayFCfollicular cyst
ddayDAB3,3'-diaminobenzidineDFdominant follicleDMIdry matter intakeE2oestradiolEDTAethylenediaminetetraacetic acidEGFepidermal growth factorELISAenzyme-linked immuno-sorbent assayFCfollicular cyst
DAB3,3'-diaminobenzidineDFdominant follicleDMIdry matter intakeE2oestradiolEDTAethylenediaminetetraacetic acidEGFepidermal growth factorELISAenzyme-linked immuno-sorbent assayFCfollicular cyst
DFdominant follicleDMIdry matter intakeE2oestradiolEDTAethylenediaminetetraacetic acidEGFepidermal growth factorELISAenzyme-linked immuno-sorbent assayFCfollicular cyst
DMIdry matter intakeE2oestradiolEDTAethylenediaminetetraacetic acidEGFepidermal growth factorELISAenzyme-linked immuno-sorbent assayFCfollicular cyst
E2oestradiolEDTAethylenediaminetetraacetic acidEGFepidermal growth factorELISAenzyme-linked immuno-sorbent assayFCfollicular cyst
EDTAethylenediaminetetraacetic acidEGFepidermal growth factorELISAenzyme-linked immuno-sorbent assayFCfollicular cyst
EGFepidermal growth factorELISAenzyme-linked immuno-sorbent assayFCfollicular cyst
ELISAenzyme-linked immuno-sorbent assayFCfollicular cyst
FC follicular cyst
5
FME fermentable metabolisable energy
FSH follicle stimulating hormone
FSHr follicle stimulating hormone receptor
G ₈ stimulatory G protein
GC granulosa cell
GDF growth differentiation factor
GGT γ glutamyl transpeptidase
GnRH gonadotropin releasing hormone
h hour
11 11001
HGF hepatocyte growth factor
HGF hepatocyte growth factor

IGFBP	insulin-like growth factor binding protein
IL	interleukin
im	intramuscular
IHC	immunohistochemistry
IU	international units
IVD	intra-vaginal device
KGF	keratinocyte growth factor
KL	kit ligand
1	litre
LC	luteal cyst
LDL	low density lipoprotein
LH	luteinising hormone
LHr	luteinising hormone receptor
mins	minutes
ml	millilitres
mmol	millimole
MMP	matrix metalloproteinase
mRNA	•
NEB	negative energy balance
NEFA	non-esterified fatty acid
ng	nanograms
NS	no-surge
NSB	non-specific binding
oFSH	ovine FSH
oLH	ovine LH
0	ovary
P4	progesterone
PAPP-A	pregnancy associated plasma protein A
PBS	phosphate buffered saline
PEG	polyethylene glycol
PG	prostaglandin
pg	picograms
PGF2a	prostaglandin F 2 alpha
PKA	protein kinase A
POF	pre-ovulatory follicle
QC	quality control
RIA	radioimmunoassay
RF	ruptured follicle
S	surge
SEM	standard error of the mean
StAR	steroidogenic acute regulatory protein
STD	standard
TC	theca cell
TC	total count

TB	total bound
TGF	transforming growth factor
TMB	tetramethylbenzidine
TNF	tumour necrosis factor
TP	total protein
VLDL	very low density lipoprotein
μl	microlitres