ULTRASONOGRAPHY AND ENDOCRINOLOGY OF OVARIAN CYSTS IN CATTLE

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor in Philosophy by Abdullahi Yusufu Ribadu.

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DECLARATION

Unless otherwise acknowledged, this thesis is all my own work, carried out in the Department of Veterinary Clinical Science & Animal Husbandry, The University of Liverpool, Leahurst, Neston, U.K. under the supervision of Dr W.R. Ward and Professor Hilary Dobson. No part of this thesis in any form, has been submitted to any other university for any other degree.

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DEDICATION

To the memory of my late father ALKALI YUSUFU RIBADU (1919 - 1988).

CONTENTS

ABSTRACT

Ultrasonography and endocrinology of ovarian cysts in cattle

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The objectives of this study were to: evaluate the detection of ovarian structures by palpation per rectum and ultrasonography compared with plasma progesterone determination; examine (by ultrasonography and plasma progesterone) the initial occurrence of ovarian cysts in postpartum cows and the response to exogenous oestradiol; monitor the response of cows with follicular cysts treated with GnRH and closely follow the development of steroid-induced ovarian cysts in heifers and the response of the heifers to exogenous oestradiol.

A close relationship was obtained between in-vitro ultrasonography and ovarian dissection for the identification and measurement of normal ovarian structures. A high correlation ($r=0.85$) was obtained between ultrasound CL diameter and plasma P_4 during the oestrous cycle in 6 cows. On days -3 and -2, however (oestrus = day 0) the CL diameter remained at mid-luteal values but was functionally inactive (progesterone < 0.5 ng/ml).

The sensitivity, specificity and positive predictive value of palpation per rectum for identifying mid-cyclic CL were 85%, 95.7% and 89.5% respectively. Ultrasonography had a sensitivity of 95%, specificity of 100% and positive predictive value of 100%.

From 29 cows presented by clinicians as having ovarian cysts (8 follicular and 21 luteal) based on palpation per rectum, only 15 (52%) were correctly diagnosed. Ultrasonography correctly determined the presence of ovarian cysts in 15 cows, large follicles (12-14 mm) in 3 cows and CL (4 with cavities) in the remaining 11 cows.

Two of 18 cows monitored during the early postpartum period developed spontaneous luteal cysts. In a further 7 cows with spontaneous follicular cysts monitored for 2 weeks, spontaneous luteinization was not observed (there was neither increase in wall thickness nor plasma progesterone). A weak correlation ($r=0.54$) was obtained between luteal tissue volume and plasma progesterone in luteal cysts. There were significantly ($P<0.05$) more follicles ($\geq$ 5 mm) in the ovary contralateral to the cyst irrespective of the type of cyst. One of 7 (14%) and 3 of 11 (27%) of cows responded to oestradiol with a surge of LH (>10 ng/ml).

Five cows with follicular cysts treated with GnRH 10 days after initial dignosis had elevated plasma progesterone (5.58 ± 0.67 ng/ml) by day 7 after treatment. Plasma progesterone on the day of diagnosis and treatment was < 0.5 ng/ml. Changes noted during weekly ultrasonography included clouding of the uniformly nonechogenic (dark) antrum of cysts, luteinization of cyst wall, reduction in cyst size (or cyst resolution) and, or, development of 1-4 CL in the ovary bearing the cyst or in the contralateral ovary.

In 6 control heifers monitored by daily ultrasonography and progesterone profile through a complete oestrous cycle, follicular dynamics were normal with 4 heifers exhibiting 2 waves of follicular growth while 2 heifers had 3 waves per cycle. Ovarian cysts were experimentally induced in 3/8 heifers using progesterone/oestradiol treatment, and in 3/7 heifers using ACTH treatment both during the late luteal phase of the oestrous cycle. A diversity of cysts (1 follicular, 2 luteal) resulted from progesterone/oestradiol treatment. ACTH-induced cysts were all follicular. A static phase was observed in all 8 heifers treated with progesterone/oestradiol before resumption of follicular growth and subsequent ovulation or cyst formation. No static phase was observed in ACTH-treated heifers. The interovulatory interval was significantly extended in both treatments, irrespective of whether ovulation or cyst formation occurred. In ACTH-treated heifers cortisol concentration significantly increased ($P<0.05$) within 24 h after beginning of treatment. Overall, 3 of 6 heifers with steroid-induced cysts responded by an LH surge to exogenous oestradiol.

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ABBREVIATION AND TRIVIAL NAMES

ACTH	adrenocorticotrohic hormone
CL	corpus(ora) luteum(a)
cm	centimetre
cortisol	4-pregnen-11ß, 17∝, 21-tiol-3, 20-diol
cpm	counts per minute
CV	coefficient of variation
d	diameter
EDTA	ethylene diaminetetra-acetic acid sodium salt
F	follicle
g	gravitational force
GnRH	gonadotrophic releasing hormone
h	hour
hcg	human chorionic gonadotrophin
ID	inner diameter
iu	international units
kg	kilogramme(s)
l	litre
LH	luteinising hormone
mg	milligramme
min	minute(s)
ml	millilitre
mm	millimetre
mCi	millicurie
ng	nanogramme
OD	outer diameter
^{0}C	degree centigrade
oestradiol (E_2)	oestra-1,3,5(10)-triene-3, 17ß-diol
PBS	phosphate buffer saline
pg	picogramme
PBS-EW	phosphate buffer saline - egg white
progesterone (P_4)	pregna-4-en-3, 20-dione
r	correlation coefficient
R.I.A.	radioimmunoassay
SD	standard deviation
SEM	standard error mean
π	pi
µg	microgramme
µl	microlitre
<	less than
>	greater than
%	per cent

CHAPTER ONE

INTRODUCTION

The development of equipment that allows ultrasound examination of the bovine genital tract has opened a new field of research (Larson, 1987). This diagnostic modality is non-invasive and, as far as is known, does not induce any biological change in the tissues nor is there apparent hazard to the operator when the equipment is properly used (Schmitz, 1991). With this technique it is possible to study in detail with minimum interference the sequential pattern of growth and regression of follicles in the ovaries throughout the oestrous cycle (Pierson and Ginther 1984; Pierson and Ginther, 1987a; Rajamahendran and Walton, 1988; Savio *et al.*, 1988; Sirois and Fortune, 1988a) and differentiate between cystic ovarian structures (Farin *et al.*, 1990; Farin *et al.*, 1992).

Previous studies on follicular and luteal changes relied on either slaughterhouse material (Morrow *et al.*, 1966) which precluded monitoring of individual animals over a period of time, or palpation per rectum (Saiduddin *et al.*, 1968) in which a measure of accuracy and precision was lost (Pierson and Ginther, 1988). Although palpation per rectum is the most widely used technique for obtaining information about normal ovarian structures and their functional status, no diagnosis is possible in some cases and a wrong diagnosis can easily be made when only one examination is carried out (Dawson, 1975).

A correct interpretation of ovarian structures such as corpora lutea (CL), follicles or cysts is essential for good diagnosis and choice of therapy (Hoffman, 1976; Belling, 1986).

Cystic ovarian disease (C.O.D.) is characterised by one or more large (>25mm) fluid-filled structures in one or both ovaries sometimes accompanied by

abnormal oestrous behaviour. It is one of the most important causes of infertility in dairy cattle (Nakao and Ono, 1977; Borsberry and Dobson, 1984).

The choice and success of therapy in C.O.D. depends on the accuracy of diagnosis. Traditionally, diagnosis of cystic ovaries is based on clinical history and findings by palpation per rectum. However, the criteria suggested in the literature to distingush the two types of cysts are confusing and poorly defined (Leslie and Bosu, 1983). For instance, although non-luteinized follicular cysts may be characterised by nymphomania or irregular oestrous cycles, and luteinized cysts by anoestrus (Roberts, 1971; Saumande *et al.*, 1979), it is also recognized that cows with either type of cysts may show variable behaviour (Bierschwall, 1966; Rankin, 1974; Saumande *et al.*, 1979). It is also often difficult to distingush or accurately classify the structures present in the ovaries during a single examination per rectum. Ultrasound examination, by accurately measuring the size and density of tissue in the ovarian cysts, may be a valuable tool to differentiate the different types of cysts and thus help in the choice of therapy.

The aims of the present study were to:

(i) evaluate the accuracy of ultrasonography in identifying and measuring normal ovarian structures by in-vitro scanning based on ovarian slicing

(ii) determine the relationship between ultrasonographic determination of corpora lutea and plasma progesterone profile in postpartum cows

(iii) compare the accuracy of palpation and ultrasonography for detecting and classifying corpora lutea based on ovarian dissection and plasma progesterone concentration

(iv) compare (using progesterone values) the accuracy of palpation per rectum and ultrasonography for the diagnosis of ovarian follicular and luteal cysts in cattle

(v) examine by ultrasonography and progesterone measurement the initial occurrence of ovarian cysts after calving and the response to exogenous oestradiol

(vi) monitor (by ultrasonography and progesterone mesasurement) clinical cases of ovarian cysts, follicular dynamics in the presence of cysts and the response to exogenous oestradiol

(vii) monitor ovarian ultrasound changes and correlate with plasma progesterone in cows with follicular cysts that have been treated with GnRH

(viii) experimentally induce ovarian cysts in heifers using progesterone/oestradiol or ACTH treatment and to monitor cyst formation and development by ultrasonography and endocrine profiles

(ix) evaluate the LH response to exogenous oestradiol in heifers with experimentally-induced cysts because cows with sponataneously occurring cysts had variable responses (Dobson and Nanda, 1992).

REVIEW OF LITERATURE

CYSTIC OVARIAN DISEASE

Definition

Cystic ovarian disease (C.O.D.); also known as cystic ovarian degeneration, cystic follicles or ovarian cysts have been defined as follicular structures of at least 25mm or more in diameter which persist for at least 10 days or more in the absence of a corpus luteum accompanied by cyclic irregularity (Roberts, 1971; Kesler and Garverick; 1982; Youngquist, 1986). Ovarian cysts are generally classified into two groups : 1) follicular cysts and 2) luteal cysts. Follicular cysts may be single or multiple, on one or both ovaries, and are usually thin-walled. Luteal cysts are generally thicker-walled than follicular cysts. Al-Dahash and David (1977) however, reported the occurrence of cystic ovaries in the presence of CL in about 30% of ovarian cyst cases encountered in an abattoir survey. The occurrence of ovarian cysts concurrently with a CL may represent situations where cystic structures remained after spontaneous resumption of oestrous cycles or response to hormonal therapy (Refsal *et al.*, 1988). It should be noted, however, that not all cows with ovarian cysts conform to this classic definition. In some cases, the size of cysts, as determined by ultrasonography, are less than 25 mm (Farin, 1993).

Ovarian cysts are anovulatory follicular structures which should be distingushed from cystic corpora lutea. A cystic corpus luteum is a corpus luteum with a central fluid cavity. Cystic corpora lutea are not pathological because they form subsequent to an ovulation and do not affect the length of the oestrous cycle (Roberts, 1971). Abnormal oestrous behaviour, varying from lack of oestrus (anoestrus) to abnormally frequent oestrus or oestrus-like activity (nymphomania)

is associated with ovarian cysts. (Seguin, 1980). Palpable uterine changes are frequently detected ranging from oedema shortly after the onset of the cystic condition to marked lack of uterine tonus as the condition becomes chronic (Seguin, 1980). There is great variation in the frequency of occurrence of the different cyst types. Zemjanis (1970) has reported that follicular cysts are much more common than luteal cysts; only 30.5% of 1190 ovarian cysts were luteal. Nessan *et al.* (1977) also recorded a higher percentage (62.6%) follicular cysts from 238 C.O.D. cows. Rankin (1974) and Dobson *et al.* (1977) however, found more luteinized cysts; 80% and 76.6% respectively.

Ovarian cysts occur primarily in dairy cows, but are occasionally detected in heifers and beef cows (Roberts, 1971). They are a common cause of reproductive failure in dairy cattle by prolonging the postpartum interval to conception (Kesler and Garverick, 1982; Leslie and Bosu, 1983).

The incidence of ovarian cysts in dairy cows has been reported to be from 5.6 to 18.8% (Bierschwal, 1966; Morrow *et al.*, 1966). The actual occurrence is probably higher because 60% of the cows that develop ovarian cysts before the first postpartum ovulation re-establish ovarian cycles spontaneously (Morrow *et al.*, 1966; Kesler *et al.*, 1979a) and may not be detected.

Aetiology

The aetiology of follicular and luteal cysts has not been conclusively determined. A commonly accepted hypothesis is that cysts result from a deficiency or mistiming in the preovulatory LH surge near the onset of oestrus, as a result of which ovulation does not occur (Seguin, 1980; Zaied *et al.*, 1981; Nanda *et al.*, 1988b). The therapeutic efficacy of LH, the histological evidence of increased

"hormone" granules and the endocrine evidence of increased concentration of gonadotrophins in the anterior pituitary of affected cows support this hypothesis (Seguin, 1980). In a study of postpartum cows that developed ovarian cysts, Kesler *et al.* (1979a) suggested that ovarian cysts developed when the hypothalamus and pituitary appeared to be less sensitive in releasing LH under the influence of endogenous oestradiol.

The cause-and-effect relationship between milk production and ovarian cysts is not well understood (Gaverick and Bierschwal, 1979). Studies on the relationship between milk yield and the occurrence of cystic ovarian disease in dairy cattle have produced conflicting results. Ovarian cysts seemed to occur more commonly in higher producing cows (Johnson *et al.*, 1966; Martin *et al.*, 1982; Barlett *et al.*, 1986). However, increased milk production may be as a result of altered hormone milieu in cows with ovarian cysts rather than a cause of ovarian cysts. Several others (Erb, 1984; Booth, 1986; Nanda *et al.*, 1989a) have concluded that C.O.D. is not particularly a disease of higher yielding cows and has no discernible effect on the milk yield or its pattern.

Principles of ultrasound

Ultrasound is defined as any sound frequency above the normal hearing range of the human ear, i.e greater than 20,000 Hertz (Rantanen and Ewin, 1981).

Diagnostic ultrasound techniques employ high frequency sound to produce cross-sectional images of soft tissues. Crystals with piezo-electric properties are electrically stimulated to produce pressure waves, referred to as "the sound beam" which is transmitted through soft tissues of the body. When the sound beam

encounters tissue interfaces of different acoustic impedance, a portion of the beam is reflected back to the transducer, which acts as a receiver. Echoes returning from soft tissue interfaces are converted to electrical impulses and displayed on a video-monitor as a cross-section. Lower frequency sound beams penetrate further into soft tissues, but have a poorer resolving capability than higher frequency sound beams (Rantanen and Ewin, 1981; Reeves *et al.*, 1984). The characteristics of a tissue determine what proportion of the sound beam will be reflected. The reflected portions appear on the ultrasound image by shades of grey extending from black to white. Liquids (e.g. follicular fluid) do not reflect sound waves (i.e they are nonechogenic or anechoic), therefore the image of a structure containing liquid appears black on the screen. At the other extreme, dense tissues (e.g. bones) reflect much of the sound beam (i.e they are hyperechogenic or hyperechoic) and appear white. Many tissues are seen in various shades of grey depending on their echogenicity (Pierson *et al.*, 1988).

Ultrasound evaluation of normal ovarian structures

Ovarian stroma: Small ovaries with no palpable structures and no visible component at slaughter are echogenic and usually brighter than most other ovarian structures. The stroma often contains numerous scattered small follicles (2-4mm diameter) which aid identification of this tissue (Edmondson *et al.*, 1986).

The corpus albicans (C.A.) may occasionally be seen on ultrasound examination as a distinct bright area measuring up to 6mm in size (Edmondson *et al.*, 1986).

Follicles/Follicular development : Pierson and Ginther (1984) first reported the use of ultrasonography to visualise structures on bovine ovaries. In a group of 5 heifers, follicles >2mm in diameter were measured and counted, but no attempt was made to follow individual follicles from day to day. It was concluded that two large follicles developed during the oestrous cycle; one during the mid-luteal phase, the other during follicular phase.

Antral follicles of various sizes appeared as nonechogenic structures which could be distinguished from blood vessels in cross-section by the elongated nonechogenic and nonspherical appearance of the latter (Omran *et al.*, 1988; Boyd and Omran, 1991).

The physiological control of recruitment, selection, growth, dominance and atresia of ovarian follicles is not clearly understood (Savio *et al.*, 1988). From abattoir specimens, Rajakoski (1960) reported on data that was consistent with two waves of follicular growth during the oestrous cycle of cattle, one between day 3 and 12 and the other between day 12 and subsequent oestrus. Donaldson and Hansel (1968) reported that growth and atresia of ovarian follicles appeared to be a continuous process, and no distinct mid-cycle waves in follicular growth were identified by palpation per rectum. A similar conclusion was reached by Mario *et al.* (1968) and Dufour *et al.* (1972). Matton *et al.* (1981) observed three waves of follicular growth in 4 groups of 10 heifers by laparoscopy and postmortem examination of ovaries.

Ovarian ultrasonography has shown that the development of bovine follicles occurs in distinct, striking and regular patterns (Sirois and Fortune, 1988b). Each wave consists of the contemporaneous emergence of a group (cohort) of follicles 5

mm or more in diameter. Within several days one follicle has grown larger than the others in the cohort and is considered 'dominant' (Ireland and Roche, 1987; Fortune *et al.*, 1988; Sirois and Fortune, 1988b; Savio *et al.*, 1988).

Savio *et al.* (1988) also observed three waves of follicular growth in 13 heifers studied by daily ultrasound examination during two complete and consecutive natural oestrous cycles. The first dominant follicle was detected by day 4, the second by day 12, while the third ovulatory follicle was identified on average by day 16. It was concluded that follicular growth is a dynamic process which is under both local and systemic control. Morphologies other than spherical were attributed to compression between adjacent follicles or a follicle and a luteal structure (Edmondson *et al.*, 1986). Pierson and Ginther (1987a) observed 58 interovulatory intervals in 22 non-pregnant Holstein heifers and found two waves of follicular growth with more follicles >2mm, 4-6mm, >13mm in the right ovary regardless of the presence of a corpus luteum.

Quirk *et al.* (1986) observed follicles >4mm during luteal regression and the follicular phase in untreated and $PGF_2\alpha$-induced luteolysis. The dynamics of development of the preovulatory follicle were similar following natural and $PGF_2\alpha$-induced luteolysis. Large non-ovulatory follicle present at the begining of luteolysis regressed during the follicular phase and the preovulatory follicle was not consistently the largest follicle present until the day of oestrus. Sirois and Fortune (1988a) observed three waves of follicle growth per cycle in 7 out of 10 Holstein heifers, with the third wave culminating in ovulation. Two out of 10 heifers had only two waves and one heifer had four waves.

The time of luteolysis relative to the time of appearance of the second follicular wave appears to determine whether cycles will have two or three waves of follicular development (Kastelic and Ginther, 1991; Fortune, 1993). The relationship between morphological dominance and functional dominance in dominant, nonovulatory follicles of the first wave was investigated by Lavoir and Fortune (1990) and Fortune *et al.* (1991). It was shown that 'nonovulatory' dominant follicles of the first wave can ovulate, while still growing, but lose functional dominance when their growth reaches a plateau. Artificial lengthening of the luteal phase in heifers by an intravaginal progesterone-releasing device; Controlled Internal Drug Release (CIDR) which maintained subluteal (low) concentration of progesterone led to prolonged follicular growth, or development of 4 or 5 follicular growth/extended cycle (Sirois and Fortune, 1990; Stock and Fortune, 1993).

Pieterse *et al.* (1990) compared the detection of follicles and corpora lutea by transvaginal ultrasonography and palpation per rectum. Ultrasonography detected 95% of follicles larger than 10mm whereas palpation per rectum detected only 71% of the follicles, as confirmed by dissection of the ovaries after slaughter. Both techniques however failed with follicles less than 5 to 10 mm in diameter as only 21.5 percent were detected by palpation per rectum and only 34.3 percent by ultrasonography.

Ovulation: By ultrasonography, ovulation is defined by the disappearence of the largest follicle in an ovary (Pierson and Ginther, 1984; Larson, 1987). This technique obviates the risk of too early rupture of the follicle. Ovulation was

detected by ultrasonography 26 to 38 h (mean 32.3 h) after onset of oestrus in 8 heifers (Larson, 1987).

Corpora lutea

The ultrasound appearance of luteal structures varies with the stage of development (Pierson and Ginther, 1984). Corpora haemorrhagica present from ovulation to day 3 after ovulation are echogenic and less dense than the mature CL. The mature CL differs from the ovarian stroma by the uniformity of its texture and the less echogenic nature (Omran *et al.*, 1988; Pieterse *et al.*, 1990). Reeves *et al.* (1984) reported the scanning of an ovary containing a cystic corpus luteum 6-8 mm in diameter. Lacunae within corpora lutea appear as nonechogenic spaces which can be differentiated from follicles by the lack of a luteal rim in the latter (Kitto *et al.*, 1986). A well defined border of the developing corpus luteum was visible after approximately day 3 (ovulation = day 0) (Pierson and Ginther, 1984; Edmondson *et al.*, 1986). Pieterse *et al.* (1990) classified corpora lutea using transvaginal ultrasonography as young (days 1 to 4), mid-cycle (days 5 to 16) or old (days 17 to 21). A young corpus luteum was a poorly defined, irregular, greyish black structure with echogenic spots, all within the contour of the ovary. A midcycle corpus luteum was a well defined granular, echogenic structure with a demarcation line visible between the corpus luteum and the ovarian stroma. In an old corpus luteum the demarcation line was faint owing to the small difference in echogenicity between the tissues. Pierson and Ginther (1984) studied the ultrasonic characteristics of the corpus luteum and stated that the echogenic pattern of the corpus luteum was

affected by the presence of central cavities in 60% of the heifers studied, which corresponded with the period of maximum size.

Accuracy of ultrasound in detecting normal ovarian structures

Pierson and Ginther (1984) found complete agreement between ultrasonography and postmortem slicing of the ovaries in location of the CL in right or left ovaries in all 23 heifers on day 12 or 14 of the oestrous cycle. Kastelic *et al.* (1990) studied the relationship between ultrasonic assessment of the corpus luteum and plasma progesterone concentration in 7 nulliparous Holstein heifers and found a high correlation between luteal tissue area and plasma progesterone concentration. Transrectal ultrasonography was more accurate than palpation per rectum for assessment of corpora lutea.

Ultrasound and ovarian cysts

Savio *et al.* (1990) defined a cystic follicle ultrasonically as a dominant follicle that exceeded 25mm in diameter for more than 10 days.

Ultrasonography of ovarian follicular cysts revealed large (25 - 55mm) nonechogenic areas that were either single or multiple large follicles of variable abnormal size, with very thin walls, while luteal cysts appeared as nonechogenic areas surrounded by echogenic tissue of varying thickness (2 - 5mm) (Edmondson *et al.*, 1986).

Omran *et al.* (1988) distingushed follicular cysts from mature follicles by size. Luteinized cysts were also differentiated from follicular cysts by the presence of a nonechogenic cyst cavity and a rim of luteinized tissue. Savio *et al.* (1990)

noted single and multiple cystic follicles in 4 out of 15 autumn-calving cows using ultrasound. Single cysts were spherical, and grew to 40 - 45mm in diameter. The walls of the cysts were less clearly demarcated than the walls of normal follicles and variable amounts of intra-follicular echogenic material were detected.

Sequential monitoring of the response of a follicular cyst in one cow to GnRH treatment was reported by Edmondson *et al.* (1986). The wall of the cyst increased in thickness from 2 mm to 6 mm over the two week period.

Accuracy of diagnosis of ovarian cysts

Classification of cysts by palpation per rectum may not always result in accurate identification of their structure and steroidogenic status (Leslie and Bosu, 1983). The findings of one or more spherical, smooth-surfaced, fluctuating structures with diameter greater than 25 mm in one or both ovaries that have persisted for at least 10 days in the absence of a corpus luteum is the basis for manual diagnosis per rectum (Bierschwal, 1966; Roberts, 1971). Palpation per rectum has been reported to be an inaccurate means for diagnosing cyst type (Seguin, 1980). The diagnosis of cyst type is mandatory for precise selection of hormonal therapy (Hoffman *et al.*, 1976).

Accuracy of diagnosis may be defined as the proportion of clinical diagnoses of follicular or luteal cysts that are confirmed by simultaneous milk or plasma progesterone determination. Booth (1986) has stated that the clinical diagnosis was considered to have been confirmed if milk progesterone concentration was less than 2.0 ng/ml for follicular cysts and 2.0 ng/ml or greater for luteal cysts. The precise cut-off point may vary between laboratories. Bierschwal *et al.* (1975) diagnosed

ovarian cysts in 144 cows (30 Guernsey and 84 Holstein) by palpation per rectum. Twenty-eight percent of the cysts were 25 to 30 mm in diameter, 60% were 31 to 40 mm and 12% were 41mm or larger. Single cysts were predominant (70%) with the highest number occurring on the right ovary. Multiple cysts were detected in 39% and occasionally involved both ovaries. No attempt was made to differentiate follicular and luteal cysts since in many instances it is clinically difficult and it was claimed that the two types of cysts may have similar response to treatment.

There is a dearth of information on the accuracy of palpation per rectum in the diagnosis of cystic ovaries. Dobson *et al.* (1977) examined 91 postpartum dairy cows with ovarian cysts; 23 were classified as non-luteinized (follicular) while 68 were classed as luteal based on palpation per rectum. Retrospective progesterone analysis revealed that 78% of the follicular cysts and 87% of the luteal cysts were correctly classified. Nakao *et al.* (1983) diagnosed follicular ovarian cysts in 160 cows based on palpation per rectum. The accuracy of diagnosis from the progesterone analysis using enzyme-linked immunoassay (ELISA) was 65%; 19% had luteal cysts while 16% had a cystic corpus luteum. The accuracy of palpation per rectum 10 days after treatment to assess luteinization of follicular cysts was only 39% based on milk progesterone analysis. The authors concluded that it was impossible to differentiate luteal cysts and cystic corpora lutea from follicular cysts by palpation per rectum. Enzyme immunoassay of milk progesterone was of practical value to differentiate follicular cysts, luteal cysts and cystic corpora lutea and to judge luteinization of follicular cysts in the cow after treatment. Nakao *et al.* (1985) also diagnosed ovarian cysts in 75 Holstein-Friesian cows by palpation per rectum. Forty-one cows had luteinized cysts while 34 were diagnosed as follicular.

The accuracy of palpation per rectum for assessing luteinization of cysts following treatment with GnRH analogue was 49% for initial luteal cases and 36% for follicular cases.

Cystic ovaries are associated with wide variation in the progesterone concentration and the significance of single hormone determinations as a diagnostic aid has been questioned, as serial progesterone determinations have revealed irregular patterns suggesting that the secretory status of the cystic structures undergoes considerable changes (Fathala *et al.*, 1978; Gunzler and Schallenberger, 1980). Despite these reservations, the determination of milk or plasma progesterone may be useful in distingushing the type of cyst and provide rationale to select the appropriate therapeutic agent.

Hoffman *et al.* (1976) reported on the classification of ovarian cysts in 186 cows according to clinical and laboratory (milk progesterone) diagnosis. In 86 cases that were clinically classified as follicular, only 47 (55%) were correctly classified based on milk progesterone concentration; 39 were luteal cysts. Similarly, in 50 cases clinically diagnosed as luteal, milk progesterone analysis revealed that 44 (88%) were correctly classified while the remaining 6 were follicular cysts. Booth (1986) however correlated 84% clinical diagnoses of follicular cysts with low milk progesterone analysis. The confirmation rate of clinical diagnosis of luteal cysts was between 50% and 60% (average 54%), almost the reverse of the results of Hoffman *et al.* (1976).

Kesler *et al.* (1978a) reported plasma progesterone concentration less than 0.1 ng/ml in 25 (78%) of 32 cows diagnosed with ovarian follicular cysts clinically. Leslie and Bosu (1983) reported that plasma progesterone concentration in 60% of

the cows diagnosed clinically as having follicular cysts was greater than that in the normal follicular phase suggesting some degree of luteinization of the cysts not detected by palpation per rectum.

The accuracy of diagnosis of follicular cysts by palpation per rectum was recorded as 58.4% by Sprecher *et al.* (1988). The predictive value of palpation per rectum for the diagnosis of follicular cysts was 75% and for the diagnosis of luteal cysts was 35% when compared with milk progesterone enzyme immunoassay (ELISA). Ax *et al.* (1986) obtained an accuracy of diagnosis of follicular cysts of 58% based on milk progesterone concentration. The milk progesterone assay may serve as a useful tool in the differential diagnosis of cystic ovaries and selection of therapeutic methods. However, for a practical use of the milk progesterone test, the assay procedure should be so simple and rapid as to be usable at the farm (Nakao *et al.*, 1983).

Hormonal Profile in Cows with Spontaneous Cystic Ovarian Disease

A substantial amount of information has been generated on the hormonal profile of cows with ovarian cysts. Concentration of progesterone in plasma is normally low in cows with follicular cysts and higher in cows with luteal cysts. The concentration of LH in plasma and pituitary in cows with follicular and luteal cysts is variable and generally higher than in normal cows (Donaldson and Hansel, 1968; Erb *et al.*, 1973). Plasma LH is, however, inversely correlated to concentration of progesterone in plasma (Kesler *et al.*, 1980). There is considerable variation in pulsatile release of gonadotrophins in cows with ovarian cysts, even among individuals with similar sex steroid profiles (Refsal *et al.*, 1988). It is not known

whether the morphological or endocrine variation seen with ovarian cysts represents stages of a single or multiple pathological mechanism (Refsal *et al.*, 1987). The concentration of plasma oestradiol in cows with follicular and luteal cysts may be higher than (Glencross and Munro, 1974; Kittock *et al.*, 1974; Cantley *et al.*, 1975) or similar to (Kittock *et al.*, 1973; Lunaas *et. al.*, 1974; Garverick *et al.*, 1976; Seguin *et al.*, 1976; Dobson *et al.*, 1977; Kesler *et al.*, 1978a; Saumande *et al.*, 1979) that in normal cows. Changes in oestrogen and plasma oestradiol observed by Lunaas *et al.* (1974) and Glencross and Munro (1974) suggest the existence of rhythmic ovarian endocrine activity in cystic ovaries. Plasma concentration of testosterone in cows with follicular and luteal cysts have been reported to be within the range observed during the oestrous cycle (Dobson *et al.*, 1977; Kesler *et al.*, 1979b; Refsal *et al.*, 1988). Dobson *et al.* (1977) suggested that cystic ovarian disease results in an abnormal time sequence of hormonal changes rather than differences in absolute values when compared with normally cycling cows. The concentration of progesterone in plasma of cows with luteinized cysts is higher than in cows with follicular cysts and there is a positive correlation between plasma progesterone concentration and luteal tissue thickness (Kesler *et al.*, 1981).

Short (1962) reported that cyst fluid contained the same oestrogens and other steroids as normal follicular fluid. Choi *et al.* (1982) determined the concentration of steroid hormones in cystic follicular fluid. High concentrations of 17α-hydroxyprogesterone, androgens and oestrogens were observed in fluid from cystic follicles with a full complement of theca and granulosa cells, while low concentrations of 17α-hydroxyprogesterone, androgens and oestrogens were found in cystic follicles without granulosa cells. Granulosa cells in ovarian cysts appear

to be as capable of producing oestradiol as normal follicles (Hansel and Fortune, 1978), which explains why a consistently high oestradiol concentration in plasma is observed in some cows without detectable follicles but with ovarian cysts (Kesler *et al.*, 1980). These different sources of oestradiol may account for the variability observed in oestradiol concentration among and within cows with ovarian cysts (Kesler *et al.*, 1980). The lack of positive relationship between concentration of oestradiol in ovarian cyst fluid and plasma (Hernandez-Ledezma *et al.*, 1982) suggests that oestradiol is also being produced by additional source(s) other than the ovarian cyst. Oestradiol production by spontaneous ovarian cysts also varied with time after cyst formation (Savio *et al.*, 1990).

Time Profile of Cystic Ovaries

Ovarian cysts appear to be dynamic structures. Cysts which occur early in the postpartum period may regress without treatment and the cow may develop a normal oestrous cycle or the cyst may be replaced by another cystic structure (Seguin, 1980). The number of cases in which spontaneous recovery occurs varies with the time postpartum at which the condition is diagnosed. As many as 60% of cows in which ovarian cysts develop prior to the first postpartum ovulation are reported to develop normal oestrous cycles spontaneously. However, spontaneous recovery occurs in only about 20% of cows that develop ovarian cysts after the first postpartum ovulation (Bierschwal *et al.*, 1975; Kesler *et al.*, 1979a; Youngquist, 1986). The phenomenon of spontaneous recovery is a confounding factor in clinical evaluation of therapeutic agents for cystic ovarian disease (Youngquist, 1986).

Kesler *et al.* (1980) monitored changes in ovaries and the concentration of reproductive hormones for 30 days in eight cows with ovarian cysts and noted changes in cystic structures and hormone secretions. Changes in progesterone concentration over the experimental period indicated luteinization of ovarian cysts in some untreated cows. Cysts originally classified as follicular may subsequently be classified as luteal. Some ovarian cysts regress in the presence of other follicular structures which subsequently develop into additional cysts. Although some luteinization does occur in untreated cysts, the degree of luteinization may not be sufficient to raise progesterone concentration to normal luteal values thus preventing re-establishment of normal ovarian cycles (Kesler *et al.*, 1977a).

The time profile of induced ovarian cysts was studied by Cook *et al.* (1990) following transvaginal ovariectomy on days 10, 20 and 40 after cyst marking. In most cases the original cyst regressed and a new follicular structure or cyst developed on the same or contralateral ovary. The turnover of cysts was accompanied by variable plasma concentrations of steroids and gonadotrophins. The high variability of peripheral concentration of LH, oestradiol and progesterone in cows with ovarian cysts across time (Cantley *et al.*, 1975; Garverick *et al.*, 1976; Kesler *et al.*, 1978a; Cook *et al.*, 1990) strongly indicates the possibility of structural and functional changes at the ovarian level. The use of ultrasound technology and endocrine assessment of cows with cystic ovarian disease will assist in monitoring these changes.

Cook *et al.* (1990) noted that the number of cows that exhibited turnover of cysts was greater than the number with spontaneous recovery or persistent cysts.

Whether the turnover rate and growth of follicles in cows with cysts is similar to that observed in cows exhibiting normal oestrous cycle is unknown.

The normal LH response to oestradiol and the failure in some cows with ovarian cysts

Injections of oestrogen in intact or ovariectomized cows consistently cause an increase in LH similar to the spontaneous rise observed at oestrus (Hobson and Hansel, 1972; Short *et al.*, 1972; Short *et al.*, 1979). A normal LH response to oestrogen in cows develops within 30 days after calving (Zaied *et al.*, 1981; Alam and Dobson, 1987). About one-third of cows in the early postpartum period (12-14 days) released LH in response to exogenous oestrogen and the LH peak was delayed compared with that in cows 30-40 days postpartum (Zaied *et al.*, 1981).

In a study on the factors affecting oestrogen-induced LH release in the cow, Short *et al.* (1979) suggested that suckling and/or lactation inhibited the oestrogen-induced LH release for at least two weeks postpartum and that there is a direct correlation between dose of oestrogen and LH response. Suckling also impairs the hypothalamic/pituitary response to low oestrogen challenge dose and changes the timing of response to high oestrogen dosage (Randell *et al.*, 1981). Berck and Convey (1977) have suggested that the oestrogen-induced LH release is apparently mediated by Gonadotropin Releasing Hormone (GnRH).

Endogenous progesterone concentration above 0.5 ng/ml prevents LH release in response to oestradiol (Hobson and Hansel, 1972; Short *et al.*, 1979; Alam and Dobson, 1987; Nanda *et al.*, 1988a). Early studies suggested that exogenous progesterone did not inhibit an oestradiol-induced LH surge (Short *et al.*, 1972) but

Nanda *et al.* (1988a) conclusively showed that the presence of ovaries is not necessary for progesterone to inhibit the surge release of LH, and that progesterone in sufficient concentration, whether endogenous or exogenous, does block the oestradiol-induced release of LH. The lack of inhibition in previous reports may be because of insufficient concentration within the hypothalamo-pituitary complex to suppress, as a result of intramuscular routes of administration elevating the plasma progesterone concentration for only a short time.

Three cows with ovarian cysts beyond 30 days postpartum released LH in response to oestradiol benzoate (2mg) but the response was delayed compared with cows 30-40 days postpartum without ovarian cysts (Zaied *et al.*, 1981). Oestradiol (1mg) completely failed to release LH in four out of four and eleven out of twelve cows with ovarian cysts in two separate studies by Dobson and Alam (1987) and Refsal *et al.* (1988). It was concluded that the hypothalamus and, or, pituitary in cows with ovarian cysts may not be as sensitive to oestradiol as in normal postpartum cows.

In a larger study of 45 cows, Nanda *et al.* (1991) reported that about 47% of cows with luteal cysts and 48% with follicular cysts did release a normal LH surge in response to oestradiol. There was no significant difference in plasma progesterone concentration at the time of oestradiol administration between responding and non-responding cows with follicular or with luteal cysts. The discrepancy in the response of cystic cows to oestradiol is not clearly understood. It has been suggested that ovarian cyst cases in which there was a response to oestradiol may represent cases in which spontaneous recovery (Seguin, 1980) may have occurred.

There seems to be a similar response pattern in cows with ovarian cysts and in those in which cysts were experimentally induced. Refsal *et al.* (1987) reported that only one of four cows with experimentally induced cysts responded with an LH surge, indicating that ovarian cysts or procedures that lead to their formation may induce the defect.

Experimental induction of ovarian cysts

Ovarian cysts have been induced in cattle by a variety of treatments given in late diestrus or proestrus. Such treatments have included exogenous oestrogens (Wiltbank, 1966; Nadaraja and Hansel, 1976), exogenous adrenocorticotrophic hormone (ACTH) (Liptrap and McNally, 1976), a combination of progesterone and oestradiol (Erb *et al.*, 1973; Winters *et al.*, 1986, Cook *et al.*, 1990; Cook *et al.*, 1991), testosterone (Fathala *et al.*, 1978) or antiserum against bovine LH (Nadaraja and Hansel, 1976). The administration of exogenous oestradiol concurrently with manual corpus luteum enucleation resulted in formation of ovarian cysts lined with grossly visible luteal tissue (Whitmore *et al.*, 1972). Ovarian cysts occurred, in 4 out of 24 lactating cows, following injection of cloprostenol 16 days after oestrus followed 12 h later by 1250 iu human chorionic gonadotropin (HCG) and 5 mg oestradiol benzoate used for oestrus synchronization (Lopez-Gatius, 1989).

In further detail, Wiltbank (1966) induced cystic ovaries (defined as one or more follicles at least 20mm in size which persisted in the absence of a corpus luteum) in 7 out 10 heifers injected with a single dose of oestradiol valerate (5 mg) late in the cycle (day 15 or 16). Nadaraja and Hansel (1976) similarly induced ovarian cysts experimentally in five out of five heifers by intramuscular

administration of oestradiol valerate (5mg) in sesame oil. The cysts produced were thin-walled and between 25 and 30 mm in diameter. Administration of 100 ml of antiserum to bovine LH subcutaneously during oestrus resulted in the formation of large and firm cysts (50-60 mm) in six out of six heifers (Nadaraja and Hansel, 1976).

Cystic ovarian follicles resulted from daily administration of ACTH during the follicular phases of the oestrous cycle in 5 non-lactating cows, the administration of hydrocortisone (cortisol) during the follicular phase of the oestrous cycle failed to induce cystic ovaries (Liptrap and McNally, 1976). Ovarian cysts were also induced in 7 out of 8 cows by twice daily injections of progesterone (0.25mg/kg) and oestradiol (0.1mg/kg) subcutaneously for 7 days (Erb *et al.*, 1973). Twice daily subcutaneous injections of 15mg of oestradiol and 37.5mg progesterone for 7 days beginning on day 15 of the oestrous cycle also resulted in the formation of cystic ovaries (Cook *et al.*, 1990; Cook *et al.*, 1991). Testosterone injection (50 mg initial dose), and increasing doses (25 mg daily up to a maximum of 250 mg) during the follicular phase of the cycle resulted in the formation of ovarian cysts in 5 non-lactating cows (Fathala *et al.*, 1978). Nadaraja and Hansel (1976) induced cystic ovaries in 6 heifers by subcutaneous administration of 100 ml of antiserum to bovine LH at oestrus.

The mechanism whereby ovarian cysts are formed after exogenous oestradiol, progesterone, ACTH or testosterone are unknown. These substances presumably interfere with the ovulatory release of gonadotrophins. The effect of exogenous oestrogen and progestins depends on the dose administered and the stage of the oestrous cycle when administered. Nadaraja and Hansel (1976) showed that

a pharmacological dose of oestradiol would cause luteal regression with a premature surge of LH that could alter follicular growth or maturation. ACTH treatment blocked the preovulatory LH surge in heifers (Stoebel and Moberg, 1982), probably through cortisol-mediated inhibition of LH release and/or increased progesterone production by adrenal glands (Stoebel and Moberg, 1982; Echternkamp, 1984; Watson and Munro, 1984). Liptrap and McNally (1976) suggested that ACTH interfered directly with the ovulatory control mechanism or stimulated production of adrenal cortical steroids (other than 11-oxygenated steroids), which blocked ovulation. The relative lack of oestradiol or progesterone release in cows forming ovarian cysts while receiving ACTH suggests suppression of gonadotrophin release (Refsal *et al.*, 1987). Overall, it is concluded that ovarian cysts may be formed either when significant gonadotrophin release occurs but is asynchronous with luteal regression and follicular maturation, or when gonadotrophin release is partially or totally inhibited.

Changes in peripheral serum oestrogen and progesterone were extremely variable among heifers in which cysts were experimentally induced by exogenous adminstration of oestrogen (Nadaraja and Hansel, 1976). Plasma concentration of oestradiol in cows with induced cystic ovaries was similar to the peak values observed at oestrus between 6 and 12 pg/ml. Progesterone concentration in plasma of cows with induced cystic ovaries was low, between 1 and 2 ng/ml (Liptrap and McNally, 1976). Plasma LH (ng/ml), progesterone (ng/ml) and oestrogen (pg/ml) in 7 cows with induced cysts were 1.7 ± 0.37, 0.37 ± 0.12 and 51 ± 9 respectively (Erb *et al.*, 1973). The proportion of steroid-induced cysts that

spontaneously recover (30%) (Cook et al., 1990) is similar to that in naturally occurring cysts (Bierschwal *et al.*, 1975).

There is variation in the proportion of animals in which ovarian cysts were succesfully induced experimentally. Refsal *et al.* (1987) succeeded in inducing ovarian cysts in 50% of cows treated with oestradiol, while Whitmore *et al.* (1972) and Wiltbank *et al.* (1961) obtained 44% and 70% success rates respectively. Nadaraja and Hansel (1976) succeeded in experimentally inducing cystic ovaries using exogenous oestrogen in 5/5 heifers and LH antiserum in 6/6 heifers.

CHAPTER TWO

GENERAL MATERIALS AND METHODS

Details of animals and specific procedures are given in relevant chapters.

Animals and Management

A total of 34 cows of unknown reproductive history were examined prior to slaughter at a local abattoir. A total of 5 visits were made to the abattoir.

A number of lactating Holstein/Friesian cows were used on 6 commercial dairy farms in Cheshire for the various studies. Cows were kept in loose housing and fed silage and commercial compounds. Water was available *ad libitum.*

The cows with spontaneous ovarian cysts were diagnosed during herd health visits based on history (no observed oestrus or irregular and/or frequent oestrus) and finding by palpation per rectum of one or more large (>25 mm) fluid-filled structures on one or both ovaries in the absence of a corpus luteum by one of five clinicians of the Farm Animal Practice, University of Liverpool. The cows were referred to this author for further investigations.

Eight heifers, 12-18 months of age and weighing 313 to 411 kg, were used for experimental induction of cysts. Heifers were kept in loose housing at the Animal Husbandry farm, Leahurst and fed hay, silage, commercial compounds and molasses. Water was available *ad libitum.*

Jugular vein catheterisation

An indwelling jugular vein catheter was inserted a day before frequent ($\leq$4 h) blood sampling. The area over the jugular vein was shaved and cleaned with a detergent-disinfectant, followed by surgical spirit. A local anaesthetic (3%

lignocaine hydrochloride: Xylotox, Willows Francis, Bolton, U.K.) was infiltrated into the skin. The vein was raised and a 14 gauge needle together with a sterile silastic catheter (ref. 602-175, ID 0.76 mm, OD 1.65 mm; Dow Corning Ltd, Reading, U.K.) was inserted into the vein. The catheter was pushed down about 10-15 cm by gentle manipulation. The needle was then removed leaving the catheter in place. The outer part of the catheter was placed in a 2.5 cm x 2.5 cm sterile adhesive tape and sutured near to the skin near to the puncture site and at a site further along the tubing length to allow free movement of head and neck.

The patency of the catheter was confirmed by withdrawing blood. The catheter was flushed with heparinised saline (500 i.u. sodium heparin/100 ml 0.9% sodium chloride) and capped (Male LUER-LOK cap, Becton-Dickinson, Rutherford, New Jersey, U.S.A.). Catheters were flushed with heparinised saline after each collection, and removed at the end of the sampling period in each experiment. About 10 ml of blood was collected using a syringe and put into a heparinised Vacutainer tube (Beckton-Dickinson, Rutherford, New Jersey, U.S.A.) on each occasion.

Tail venepuncture

Infrequent blood samplings were carried out by coccygeal venepuncture (10 ml) into heparinised Vacutainers. All blood samples were centrifuged at 1000 g for 10 min and plasma stored in plastic vials at -20^{0}C until analysis.

Ultrasonography

Ultrasonography was carried out using an ultrasound scanner (Aloka Echocamera SSD 210 DXII, Livingstone, Scotland) equipped with a 7.5 MHz rectal

transducer (Aloka, UST 55111, Japan). Ultrasound scanning was performed by the same operator throughout this study. Faecal material was removed from the rectum before examination. A coupling gel was placed on the transducer before insertion into the rectum. Each ovary was scanned in several planes by moving the transducer along the surface of the ovary in order to identify follicles, corpora lutea or cysts. Maximum diameter of each structure was measured on each occasion using the in-built electronic calipers after freezing the image on the screen. Representative images were printed using a thermal printer (Sony, Video Graphics UP-850, Japan).

Radioimmunoassay of hormones

Progesterone

Plasma progesterone was measured using an antiserum raised against 11α succinyl progesterone in rabbits at a working dilution of 1:8000 (Kanchev *et al.*, 1976). Plasma samples and progesterone standard at 0, 20, 50, 100, 200, 300 and 500 pg/100μl were analysed in duplicate. Extraction of 0.1 ml plasma was done in 1 ml hexane. The extract was evaporated to dryness and incubated with 0.1 ml of antiserum and 0.1 ml tritiated progesterone (16000 dpm) at 4 ^{o}C. After 12-18 h, antibody-bound and unbound hormones were separated by incubating with 0.5% charcoal suspension for 10 min and centrifuging at 3000 g for 10 min. The supernant was decanted, 2 ml of Optiphase$^{(R)}$ (LKB, FSA laboratory Supplies, Loughborough, England) scintillation fluid added and the activity counted in a beta counter (Tri-Carb 1900 TR Liquid Scintillation Analyser, Packard, Canberra Co., Meriden, USA).

Cortisol

The antiserum used was that raised in rabbits against hydrocortisone-3-0-carboxymethyloxime-BSA (Sigma) by Alam *et al.* (1986). This procedure was a modification of that method.

Briefly, duplicates of plasma or standards in ethanol at 0, 20, 50, 100, 300, 500 and 1000 pg/tube were analysed. Extraction of 0.01 ml plasma was achieved by vortexing with 1 ml ethanol for 30 sec and centrifuging at 3000 g for 10 min. The supernatant was decanted, evaporated and incubated with 0.1 ml antiserum (1:10,000) and 0.1 ml tritiated cortisol (20000 dpm). Bound and unbound hormone was separated by addition of the scintillator fluid Optiscint$^{(R)}$ and freezing out the aqueous phase containing bound hormone in an alcohol cooling bath (Fryka, KB 300, Camlab Ltd, Cambridge, England). The supernatant (free hormone) was decanted into a plastic counting vial and activity counted in the beta counter.

Estimation of plasma LH concentration

A double antibody radioimmunoassay (RIA) using iodinated LH characterised and verified in this laboratory was used (Alam and Dobson, 1986).

Iodination procedure

A sephadex G50 column (ID 6 mm) was rinsed with 1.5 ml phosphate buffered saline with 1% egg white (PBS-EW) and then eluted with PBS for 10 min leaving 2 cm on the top of the column. Chloramine-T (20 mg/10 ml) and sodium metabisulphite (25 mg/10 ml PBS) solutions were freshly prepared. An aliquot of frozen transfer-solution (1g potassium iodide and 10 mg bromophenol blue diluted to 100 ml with 16% sucrose solution) was thawed. One mCi ^{125}I and 2.5 μg purified

LH in 2.5 μl PBS-EW in Durham tube were mixed gently for 60 sec. The reaction was stopped with 50 μl sodium metabisulphite solution. Transfer solution, 0.1 ml, was added to the reaction vessel and transferred to the column which was then continuously eluted with PBS; 0.5 ml portions were collected in tubes containing 0.5 ml PBS-EW. Radioactivity in the eluates was assessed, those containing the first peak of radioactivity were stored at 4^0C for subsequent use. The second peak, containing free iodine, concomitant with bromophenol, was discarded. The iodinated LH was used within 2 weeks.

R.I.A procedure for LH

Disposable LP/3 plastic tubes (Luckman Ltd, Sussex, U.K.) were used. Plasma duplicate (0.1 ml) or bovine LH standards (NIH-B8) in PBS-EW in triplicates of 0, 0.05, 0.5, 1.0, 3.0, 5.0 and 7.5 ng/0.1 ml were incubated with 0.1 ml 1:12,000 antibody in normal rabbit serum diluted with 0.05M PBS-EDTA (1:300) for 24 h at 4^0C. Next day, 0.1 ml ^{125}I-LH (40,000 dpm) was added, vortexed and again incubated for 24 h at 4^0C. To separate bound hormone from the unbound, 0.1 ml second antibody solution (1:50 donkey anti-rabbit serum; IDS Boldon, Tyne & Wear, England) was added and incubated for 24 h. The following day, 1 ml ice-cold water was added and spun at 3000 g for 30 min in a refrigerated centrifuge. The supernatant was drained and pellets were counted for the radioactivity.

Activity of ^{125}I was counted for 1 min in a gamma counter (LKB Wallac Rackgamma II, Finland).

Extraction : In each assay of steroid hormones, a known amount of radioactive hormone was added to plasma. Extraction efficiency was estimated by the recovery of the added hormone (Table 3.1). Plasma concentration in the samples was corrected for extraction losses.

Specificity : The specificities of the antisera used for radioimmunoassay of various hormones is given in Table 3.2 and the binding at zero point and non-specific bindings are given in Table 3.3.

Sensitivity : Expressed as the value 2 S.D. below the zero point of the standard curve (Table 3.3).

Table 3.1: Extraction recovery (mean $\pm$ S.D.), intra and inter-assay coefficients of variation (C.V., %) for radioimmunassay of various hormones.

Hormone	Extraction recovery (%)	Intra-assay C.V. (%)	Inter-assay C.V. (%)
Progesterone	64.9 $\pm$ 1.8	4.6	8.7
Cortisol	87.7 $\pm$ 2.0	6.0	12.7
LH	-	11.6	13.5

Table 3.2: Specificities of antibodies used in radioimmunoassay of various hormones

Hormone	Cross-reaction with hormone congeners	Reference
Progesterone	11∝-hydroxyprogesterone = 64%	
	11ß-hydroxyprogesterone = 72%	Dobson (1978)
	Other steroids = < 2%	
Cortisol	Cortisone = 8.4%	
	Corticosterone = 8.3%	Alam *et al.*
	11-deoxycorticosterone = 0.9%	(1986)
	Other steroids = < 0.01%	
LH	Thyroid stimulating hormone = 30%	Alam and Dobson (1986)

Table 3.3: Sensitivity, specific and non-specific bindings (%) in radioimmunoassay of various hormones

Hormone (number of assays)	Sensitivity/ml	Non-specific binding	Binding at zero point
Progesterone (30)	50 pg	2.83 ± 0.76	42.4 ± 2.80
Cortisol (5)	100 pg	0.82 ± 0.91	43.9 ± 4.80
LH (3)	500 pg	1.97 ± 0.08	52.8 ± 3.90

CHAPTER THREE

RELIABILITY OF ULTRASONIC SCANNING OF COW OVARIES

INTRODUCTION

Ultrasonography is a non-invasive diagnostic technique that uses reflection of high frequency sound waves from tissue interfaces to produce visual images.

Only a few studies have assessed the accuracy of ultrasonography in evaluating ovarian structures in cows. There was a linear relationship between follicle diameter measured by in vivo ultrasonography and follicle diameter determined after slaughter (Driancourt *et al.*, 1991). Correlation coefficients of 0.8-0.9 for various sizes of structure between in vivo ultrasonography and postmortem slicing of excised ovaries have been reported (Pierson and Ginther 1988; Pierterse *et al.*, 1990; Driancourt *et al.*, 1991). The practice of scanning ovaries from slaughter-house reproductive organs in a water bath may be helpful to learn identification of ovarian structures and interpretation of the two-dimensional ultrasound images.

The objective of the present study was to evaluate the accuracy of ultrasonography by in-vitro scanning followed by slicing of excised ovaries to identify and measure ovarian structures of cows.

MATERIALS AND METHODS

The genital organs of 25 cows of unknown reproductive history were obtained from a local abattoir. Ovaries were fixed in 10% neutral buffered formalin for 12 hours to prevent collapse of the follicles on slicing (Pierson and Ginther, 1987b). Each of the ovaries was scanned in a waterbath using a real-time B-mode

diagnostic ultrasound scanner (Concept One, Dynamic Imaging, Livingstone, U.K.) equipped with a linear array 7.5 MHz transducer. Follicles in various diameter categories (2-5mm, 6-10mm, 11-15mm and 16-20mm) were counted and the diameter of follicles $\geq$6mm were measured with in-built calipers after freezing the ultrasound image on the console video screen. The presence of corpora lutea and corpora albicantia was also recorded and diameters determined. Each of the ovaries was sliced in a plane similar to the ultrasound scan and the follicles and corpora lutea were counted and measured using a vernier caliper (Camlab, Cambridge, U.K.). The slicing results were recorded separately. The two types of measurement were compared statistically using Student's *t* test, correlation and regression analyses.

RESULTS

The mean number $\pm$ S.D and mean diameter $\pm$ S.D of follicles and corpora lutea in various diameter categories is given in Tables 1 and 2.

For the various sizes of follicles categories the sensitivity of ultrasonography, defined as the proportion of correctly identified follicles and corpora lutea from slicing measurements of the ovaries were :- 2-5mm (95.62%), 6-10mm (95.83%), 11-15mm (92.30%), 16-20mm (100%), CL (100%), and regressing corpora lutea (33.33%).

There was no significant difference ($P > 0.05$) between ultrasonography and slicing of the ovaries for any size of follicles or CL. There was a linear relationship between ultrasonography and slicing measurement of the ovaries for the sizes of the follicles or CL. The regression equations were:-

6-10mm : y = 0.9X + 1.236 (r, 0.90), 11-15mm : y = 0.913X + 1.808 (r, 0.94), 16-20mm : y = 1.95X - 14.95 (r, 1), CL : y = 0.9X + 1.7 (r, 1), where y was diameter (mm) determined by slicing of the ovaries and X the diameter obtained by ultrasonography.

Follicles appeared ultrasonographically as round, circumscribed, nonechogenic (black) structures while mature corpora lutea were well-defined, granular echogenic structures.

Table 1: Mean number ± S.D of follicles in various diameter categories as determined by in vitro ultrasonography and slicing (n=50 ovaries)

Size of folliles	Ultrasonography	Slicing
2-5mm	5.94 ± 2.20	5.68 ± 2.92
6-10mm	0.48 ± 0.76	0.46 ± 0.73
11-15mm	0.26 ± 0.49	0.22 ± 0.42
16-20mm	0.04 ± 0.20	0.04 ± 0.20

Table 2: Mean diameters (mm) ± S.D of follicles in various diameter categories and corpora lutea as determined by in vitro ultrasonography and slicing (n=50 ovaries)

Size of follicles	Ultrasonography	Slicing
6-10mm	7.6 ± 1.7	8.0 ± 1.7
11-15mm	12.5 ± 1.5	13.2 ± 1.5
16-20mm	16.5 ± 0.7	17.2 ± 1.4
CL	15.6 ± 6.1	15.6 ± 5.6

DISCUSSION

The high correlation ($r = > 0.9$) between in-vitro ultrasonography and ovarian dissection in detecting ovarian structures confirmed this as a highly accurate method of determining normal ovarian structures. The correlation appeared to increase with size of the follicles. There was complete agreement ($r=1$, $P<0.05$) between ultrasonography and slicing measurement in identification of mature corpora lutea and corpora lutea with cavities. A similar result was obtained by Pierson and Ginther (1987b). Cavities within the corpora lutea were observed as nonechogenic spaces (<20mm) in diameter surrounded by a rim of luteal tissue.

Ultrasonographic measurement of the diameters of follicles $\geq$6mm was slightly less accurate than slicing measurement, though not significantly different ($P>0.05$). This difference can be accounted for by the fact that ultrasound measurement in this study was essentially of antral diameters (excluding the follicular wall) while slicing measurements included the follicular wall. During ultrasonography, the demarcation between the antrum and inner follicular wall is more clearly defined than that between the outer follicular wall and ovarian stroma. It is generally agreed that follicles measure 2 to 3mm larger on dissection than they appear on scanning (Quirk *et al.*, 1986; Sirios and Fortune, 1988b).

Ultrasonography detected only 4 out of 12 old CL (33.3%). Pieterse *et al.* (1990) similarly recorded only 4 of 11 old CL by ultrasonography. There is little difference in the tissue density of old corpora lutea and ovarian stroma, hence little difference in echogenicity. Ultrasonography is therefore not reliable in detecting old CL. A dense echogenic line was noted in the centre of some CL which was not

visible on slicing. A similar observation was made by Omran *et al.* (1988) and M.A.M. Taverne (1991; personal communication).

In conclusion, ultrasonography was a reliable, accurate and objective technology for identifying and measuring ovarian structures in cows.

CHAPTER FOUR

COMPARATIVE EVALUATION OF OVARIAN STRUCTURES IN CATTLE BY PALPATION PER RECTUM, ULTRASONOGRAPHY AND PLASMA PROGESTERONE DETERMINATION

INTRODUCTION

Several studies have compared the accuracy of diagnosis of corpora lutea (CL) by palpation per rectum based on progesterone values. However, very few have studied the accuracy of palpation and ultrasonography by comparison with gross ovarian dissection and progesterone concentration.

Quirk *et al.* (1986) stated that decreases in the sizes of CL in heifers were coincident with decreases in plasma concentration of progesterone. Sprecher *et al.* (1989) obtained a correlation coefficient of 0.68 between radioimmunoassay (milk) progesterone and ultrasonographic CL diameter. Kastelic *et al.* (1990) concluded that ultrasonographic assessment was a viable alternative to determination of peripheral progesterone concentration for evaluation of luteal function.

Plasma progesterone concentration and palpation per rectum of ovaries for CL have an agreement of 77-87% (Boyd and Munro, 1979; Ott *et al.*, 1986; Archbald *et al.*, 1992). A similar correlation was obtained by Watson and Munro (1980) between palpation and milk progesterone.

Kähn and Leidl (1986) compared palpation and transrectal ultrasonography (5MHz transducer) for identifying CL with ovarian dissection, and obtained a sensitivity of 85% for both methods. A 100% agreement was reported between in vivo ultrasonography and ovarian dissection postmortem for the identification of CL and CL with cavities (Pierson and Ginther, 1987b). Pieterse *et al.* (1990) reported

sensitivity and predictive value of palpation for detecting mid-cycle CL as 83.3% and 73.2% and of transvaginal ultrasonography (5MHz transducer) as 80.6% and 85.3% respectively.

The diagnosis of an ovarian cyst in cattle is usually based on reproductive history and palpation of a smooth fluid-filled structure greater than 25mm in diameter in one or both ovaries in the absence of a CL (Bierschwal *et al.*, 1975). Correct choice of therapy for cystic ovarian degeneration depends on accurate diagnosis - a long recognized problem facing veterinarians (Dobson and Nanda, 1992).

The accuracy of palpation for ovarian cyst diagnosis is usually tested by comparison with progesterone measurement in peripheral plasma or milk. A wide variation of accuracy has been quoted by different authors; 55-84% for follicular cysts and 54-88% for luteal cysts (Booth 1976, Hoffman *et al.*, 1976, Dobson *et al.*, 1977).

Sprecher *et al.* (1988) reported the positive predictive value, sensitivity and specificity of diagnosis by palpation as 75.0%, 61.9% and 50% respectively for follicular cysts and 35.1%, 50% and 61.9% respectively for luteal cysts. It was concluded that the predictive value of palpation for diagnosis of follicular cysts was too low for specific therapeutic decisions. It has recently been reported that the use of on-farm milk progesterone kits did not appear to improve the diagnosis rates (Dobson and Nanda, 1992).

The use of ultrasonography for the diagnosis of ovarian cysts in cattle has been documented (Edmondson *et al.*, 1986; Kähn and Leidl, 1989; Carroll *et al.*, 1990, Farin *et al.*, 1990; Farin *et al.*, 1992). There is scanty information however

on the diagnosis of ovarian cysts by palpation, ultrasonography and plasma progesterone determination.

The aims of the present study were:-

(1) to determine the relationship between ultrasonographic determination of CL and plasma progesterone profile in postpartum cyclic cows

(2) to compare the accuracy of palpation and ultrasonography for detecting and classifying corpora lutea based on ovarian dissection and plasma progesterone concentration

(3) to compare (using progesterone values) the accuracy of palpation per rectum and ultrasonography for the diagnosis of ovarian follicular and luteal cysts in cattle.

MATERIALS AND METHODS

Ultrasonography was carried out using an ultrasound scanner (Aloka Echocamera SSD 210 DXII, Livingstone, Scotland) equipped with a 7.5 MHz rectal transducer (Aloka, UST 55111, Japan) as described in chapter 2. Classification of CL into developing, mid-cycle and regressing depended on their ultrasound appearance. A developing CL was a poorly defined, irregular, greyish-black structure with echogenic spots all within the ovary; a mid-cycle CL was a well defined granular, greyish echogenic structure with a demarcation line visible between the CL and the ovarian stroma; in a regressing CL the demarcation line was faint owing to the slight difference in echogenicity between the tissues (Pieterse *et al.*, 1990).

A blood sample (10 ml) was obtained from each cow by tail venepuncture, centrifuged at 1000g and stored at -20°C until analysis. Plasma progesterone was

determined using radioimmunoassay (Kanchev *et al.*, 1976). The characteristics of this assay is given in chapter 2.

Corpora lutea diameter and progesterone concentration were correlated using Pearson's correlation (Snedecor and Cochran, 1980). Sensitivity, specificity and predictive value of palpation and ultrasonography were calculated according to Sprecher *et al.* (1989).

Study 1: Six cyclic postpartum cows were scanned three times a week for one month. Blood was obtained after each examination for progesterone analysis. Oestrus detection was carried out three times a day by observation of mounting behaviour. As the length of the oestrous cycle varied in the six cows, the data was standardised through one complete cycle by taking day 0 as the day of oestrus; days 1-10 as the days following oestrus and days -9 to -1 as the days preceding oestrus.

Study 2: Ovaries of 34 cows (Friesian/Holstein) of unknown reproductive history were palpated, scanned per rectum and dissected post-mortem in order to detect and classify corpora lutea into developing (days 1-4), mid-cycle (days 5-16) or regressing (days 17-21) according to morphological criteria (Zemjanis, 1970). A developing CL by palpation per rectum was a small, soft structure on the surface of the ovary; a mid-cycle CL was a more defined structure which sometimes protruded from the surface of the ovary; a regressing CL was a structure which protruded from the surface of the ovary less than at mid-cycle, but which was more compact and almost as firm as the ovarian stroma. The presence of large follicles $\geq$10mm were also noted. An identification number in a polythene sheet was inserted into the vagina of each cow for identification of the genitalia after slaughter.

Genitalia were recovered after slaughter and immediately transported to the laboratory. CL and large follicles were dissected out, and measured using vernier calipers. CL were classified into developing, mid-cycle or regressing according to their gross morphological appearance. In addition mid-cycle CL were also weighed. All examinations (palpation and ultrasonography) and ovarian dissection were carried out by the same operator.

Study 3: Four experienced clinicians presented 29 cases of cystic ovaries after regular fertility visits to dairy farms. Diagnosis of an ovarian cyst by the clinicians was based on reproductive history (nymphomania, short or irregular oestrous cycles or anoestrus) and palpation of large fluid-filled ovarian structure(s) on the ovarian surface in the absence of a CL. Cysts were further classified into follicular (thin-walled) or luteal (thick-walled).

Ultrasound diagnosis of ovarian cysts was based on the presence of a nonechogenic area $\geq$25mm diameter on the ovaries. Classification of cysts into follicular (uniformly nonechogenic antrum, with thin wall $\leq$3mm) or luteal (nonechogenic antrum with grey patches within the antrum or along the inner cyst wall) with a wall thickness >3mm was based on the appearance of the ultrasonograms.

RESULTS

Study 1: The mean length ($\pm$ S.E.M) of the oestrous cycle in the 6 cows was 21.30 $\pm$ 0.21 days. The relationship throughout the oestrous cycle between CL diameter and plasma progesterone concentration is given in Fig 4.1. A corpus luteum was clearly detectable by ultrasonography from day 5. A CL appeared as a

granular, echogenic structure with well defined borders (Fig 4.2). A cavity at the centre of the CL was observed in one cow (cow 4). The maximum diameter of the cavity was 16mm on day 10 of the cycle. The cavity appeared ultrasonographically as a non-echogenic (dark) area surrounded by the echogenic luteal tissue.

A high correlation (r = 0.85) was obtained between CL diameter and plasma progesterone concentration with most discrepancy on days 18-21.

Study 2: There was complete agreement between classification of CL based on ovarian dissection and plasma progesterone concentration, which were used as the standard for assessing palpation and ultrasonography. Classification of CL by palpation, ultrasonography, dissection and progesterone is presented in Table 4.1.

No developing CL was found by any method. The specificity, sensitivity and positive predictive values of ultrasonography for identifying midcycle CL were higher than those of palpation (Table 4.2). Plasma progesterone concentration (mean $\pm$ S.E.M) in cows with midcycle CL (n=20) was 6.13 $\pm$ 0.67 ng/ml while in cows with old CL (n=3) and without CL (n=11) were 0.22 $\pm$ 0.11 ng/ml and 0.10 $\pm$ 0.02 ng/ml, respectively. Mid-cycle CL weighed between 2.17 and 8.86gm. Ultrasonography detected double CL (n=3) and CL with cavities (n=2) which were subsequently confirmed by dissection. Of 3 regressing CL (associated with low P_4), only one was detected by ultrasonography while one was wrongly classified by palpation as a mid-cycle CL. There was a linear relationship between CL weight and plasma progesterone concentration with a correlation coefficient of 0.87.

Palpation detected only 7/25 (28%) of follicles $\geq$10mm while ultrasonography identified 19/25 (76%) of the follicles found by dissection.

Study 3: Classification of ovarian cysts based on palpation per rectum and ultrasonography is given in Tables 4.3 and 4.4, while Figs 4.3-4.6 are representative ultrasonograms of a follicular cyst, a luteal cyst, a large follicle and a CL with a cavity. The raw data of the cases presented is provided as appendix IA.

Overall, palpation correctly diagnosed ovarian follicular and luteal cysts in 15 out of 29 cows (52%). Ultrasonography revealed the presence of ovarian cysts in 15 cows (6 follicular and 9 luteal), large follicles (diameter 12-14mm) in 3 cows and CL (with or without cavities) in the remaining 11 cows. Mean plasma ($\pm$ S.E.M.) progesterone was 0.1 $\pm$ 0.03 ng/ml for cows with follicular cysts (n=5), 3.6 $\pm$ 0.49 ng/ml for cows with luteal cysts (n=10) and for the 3 cows with large follicles and 11 cows with CL 0.46 $\pm$ 0.05 ng/ml and 6.15 $\pm$ 0.50 ng/ml, respectively. In 11 cases (79% of misdiagnosis) a CL was diagnosed as a luteal cyst; in 3 cases (21% of misdiagnosis) a large follicle was diagnosed as a follicular cyst by palpation. In one case (7% of misdiagnosis) a luteal cyst was diagnosed as a follicular cyst by ultrasound. Ultrasonographic classification of cyst type was consistent with progesterone classification in 14 out of 15 (93%) cases.

Fig. 4.1: Ultrasound determination of corpora lutea diameter (—□—) and plasma progesterone concentration (—●—) in 6 cows (mean ± s.e.m)

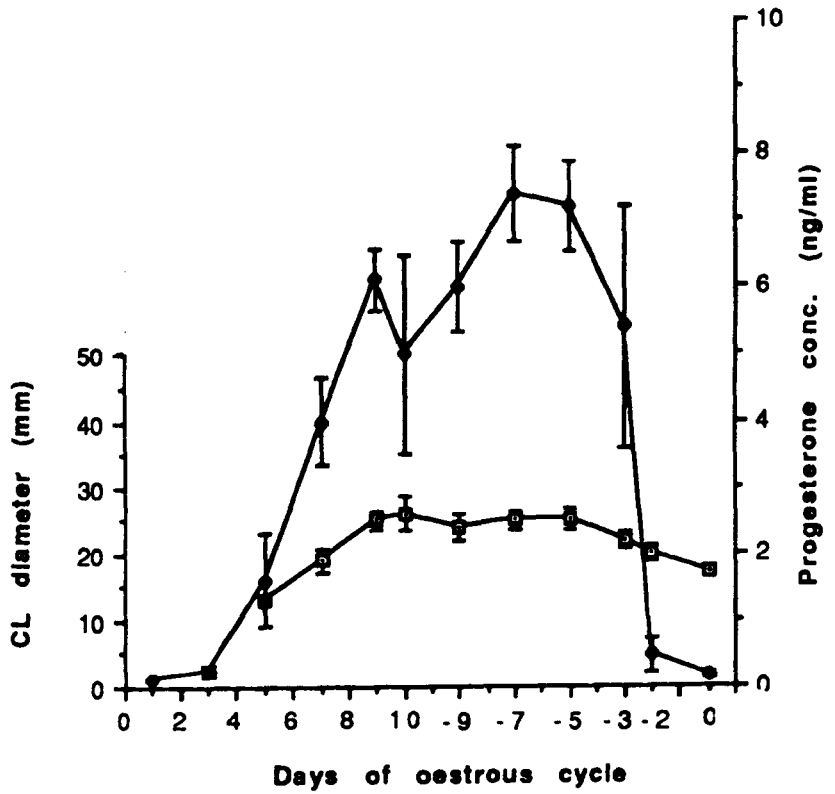


Table 4.1: Classification of corpora lutea by palpation per rectum, ultrasonography based on dissection.

CL stage	Dissection	palpation	Ultrasound
Developing	0	0	0
Mid-cycle	20*	17	19*
Regressing	3	0	1

*3 double CL were each counted as 1 unit.

Table 4.2: Sensitivity, Specificity and Positive predictive value of palpation per rectum and ultrasonography for identifying mature CL.

a) Palpation per rectum

	CL+ found	CL- not found
CL+ present	17 (a)	2 (b)
CL- not present	3 (c)	46 (d)

Sensitivity = a/(a+c)x100 = 17/20 x 100 = 85%
Specificity = d/(b+d)x100 = 46/48 x 100 = 95.8%
Positive predictive value = a/(a+b)x100 = 17/19 x 100 = 89.5%

b) Ultrasonography

	CL+ found	CL- not found
CL+ present	19 (a)	0 (b)
CL- not present	1 (c)	48 (d)

Sensitivity = a/(a+c)x100 = 19/20 x 100 = 95%
Specificity = d/(b+d)x100 = 48/48 x 100 = 100%
Positive predictive value = a/(a+b)x100 = 19/19 x 100 = 100%

Table 4.3: Classification of ovarian "cysts" based on palpation per rectum and plasma progesterone profile

Method	Progesterone	classification	
Palpation diagnosis	low <0.9ng/ml	high >0.9ng/ml	Total
Follicular cyst	8	0	8
Luteal cyst	0	21	21
	8	21	29

Table 4.4: Classification of ovarian cysts and other structures by ultrasonography and progesterone profile

Method	Progesterone	classification	
Ultrasound diagnosis	low <0.9ng/ml	high >0.9ng/ml	Total
Follicular cyst	5	1	6
Luteal cyst	0	9	9
Large follicle	3	0	3
Corpora lutea	0	11	11
	8	21	29

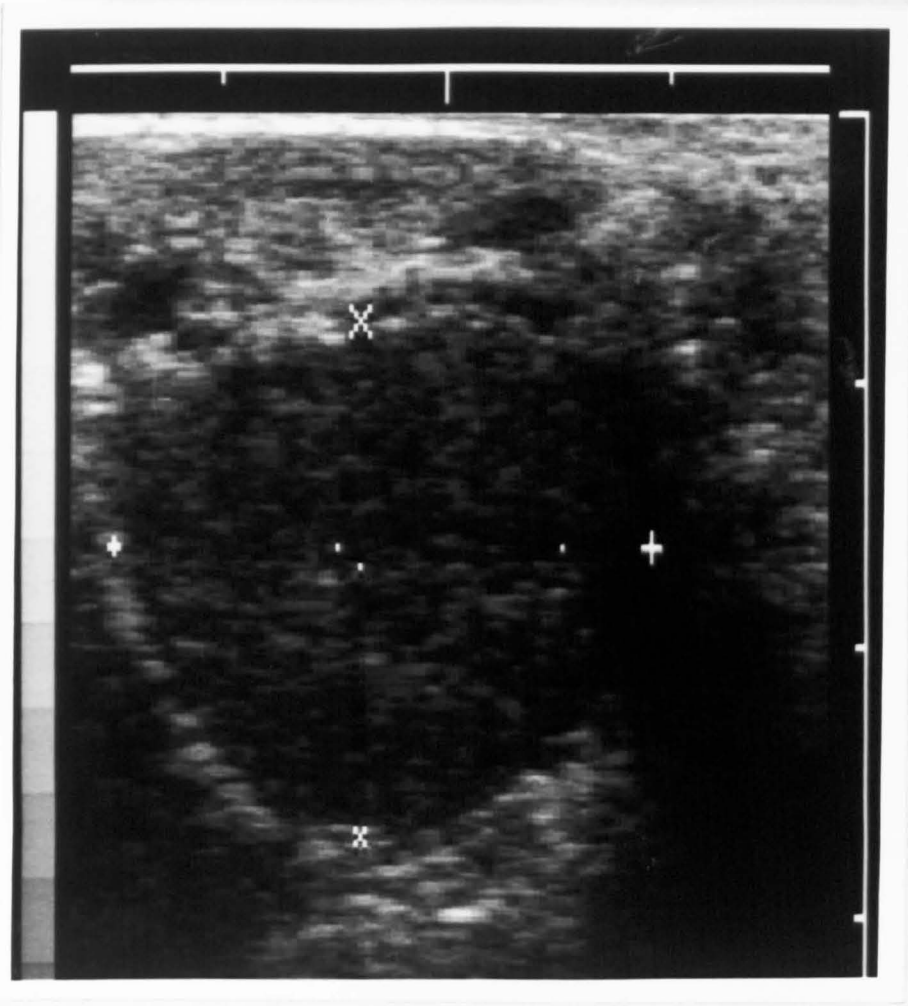


Fig 4.2 : Ultrasonogram of a corpus luteum

Size of CL = 24 x 19 mm

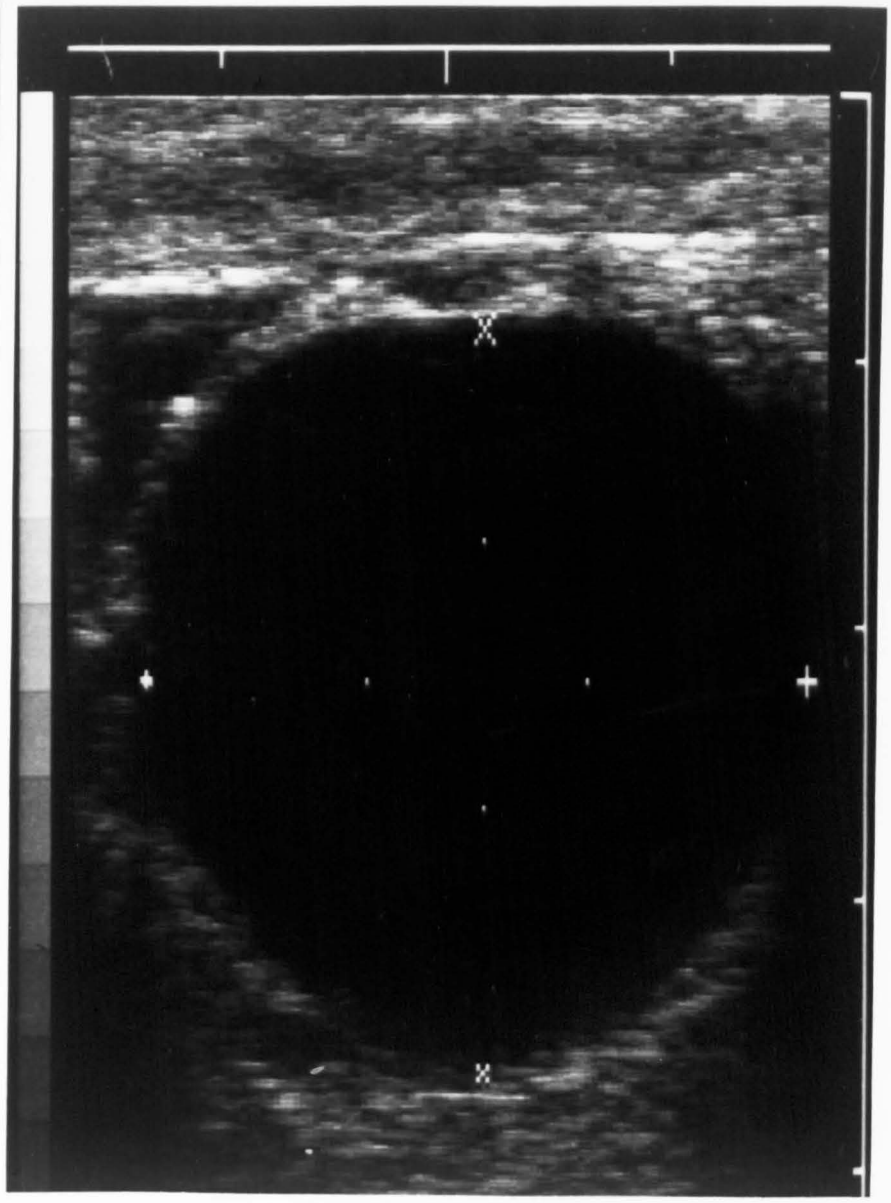


Fig 4.3: Ultrasonogram of a follicular cyst

Cyst cavity diameter = 29mm

Cyst diameter = 31mm

Progesterone concentration = 0.17 ng/ml

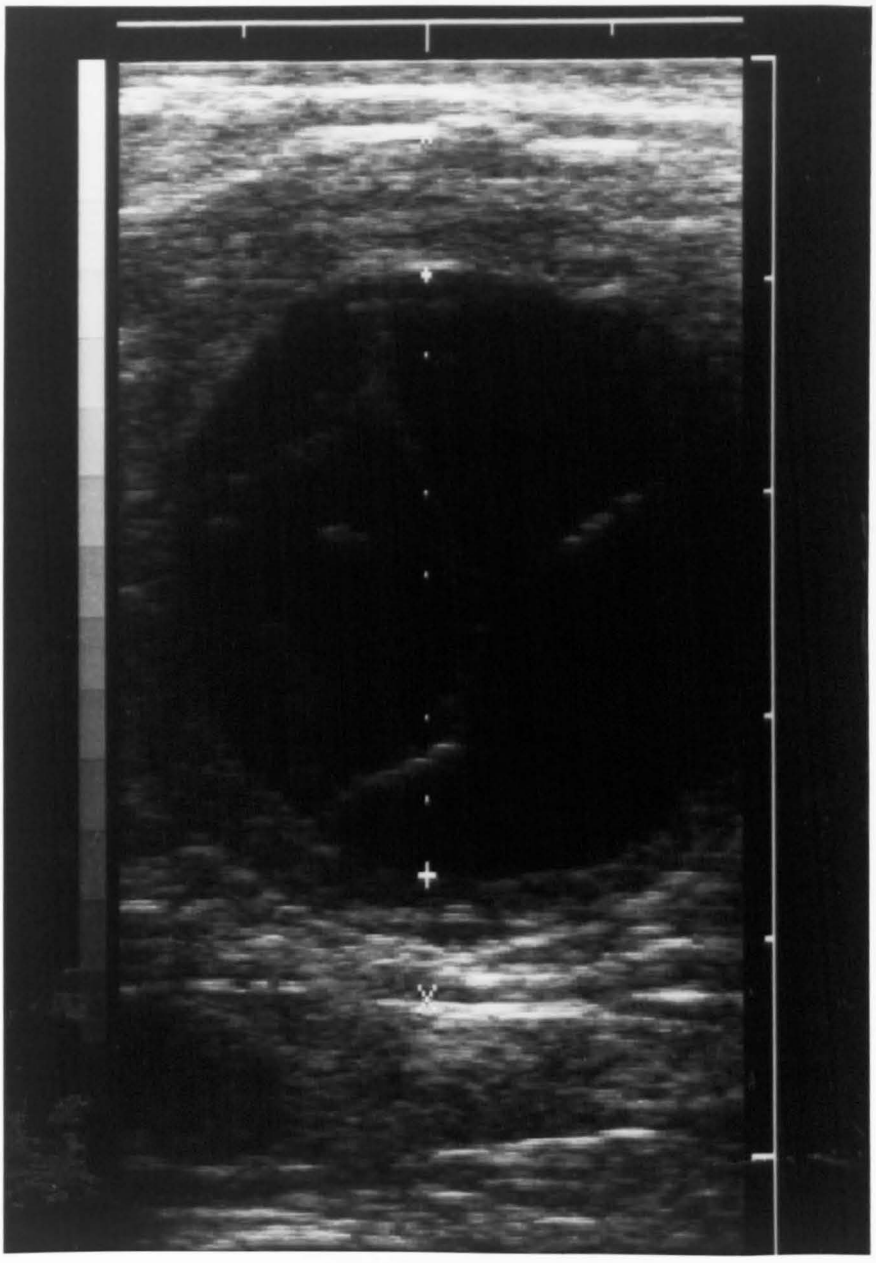


Fig 4.4: Ultrasonogram of luteal cyst

Cyst cavity diameter = 26mm

Cyst diameter = 36mm

Progesterone concentration = 2.72 ng/ml

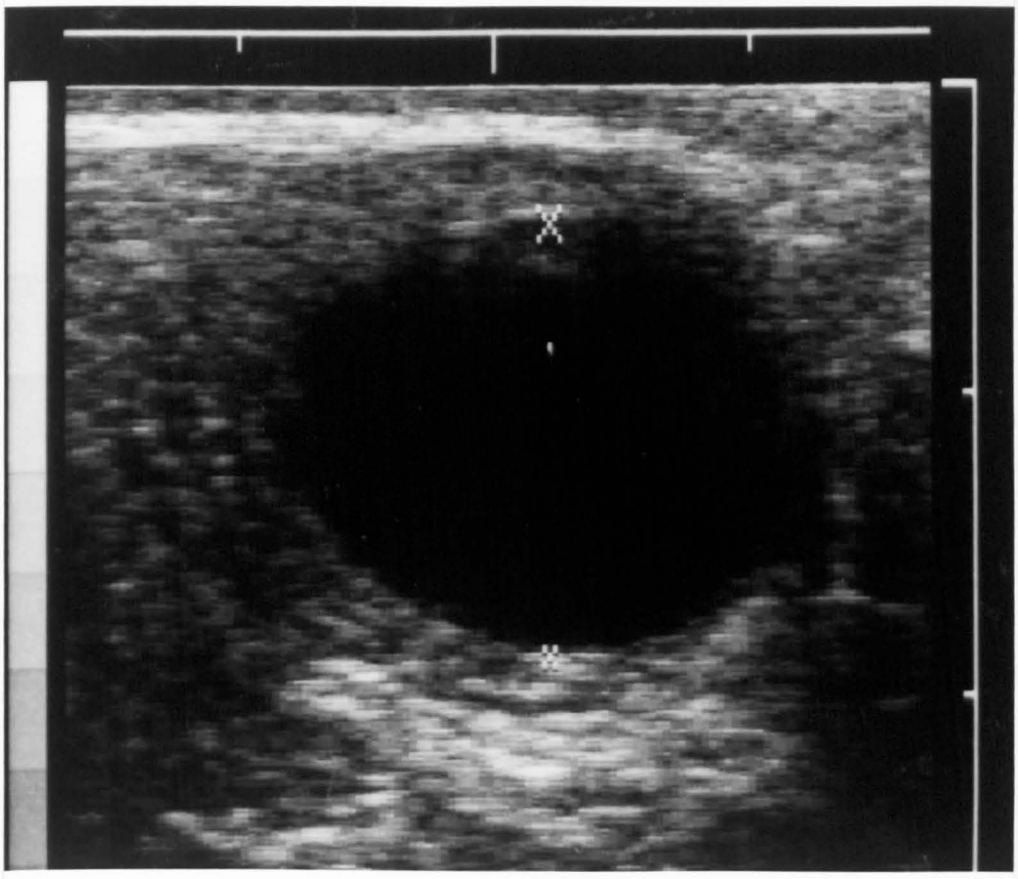


Fig 4.5: Ultrasonogram of a large follicle

Follicle diameter = 14mm

Progesterone concentration = 0.36 ng/ml

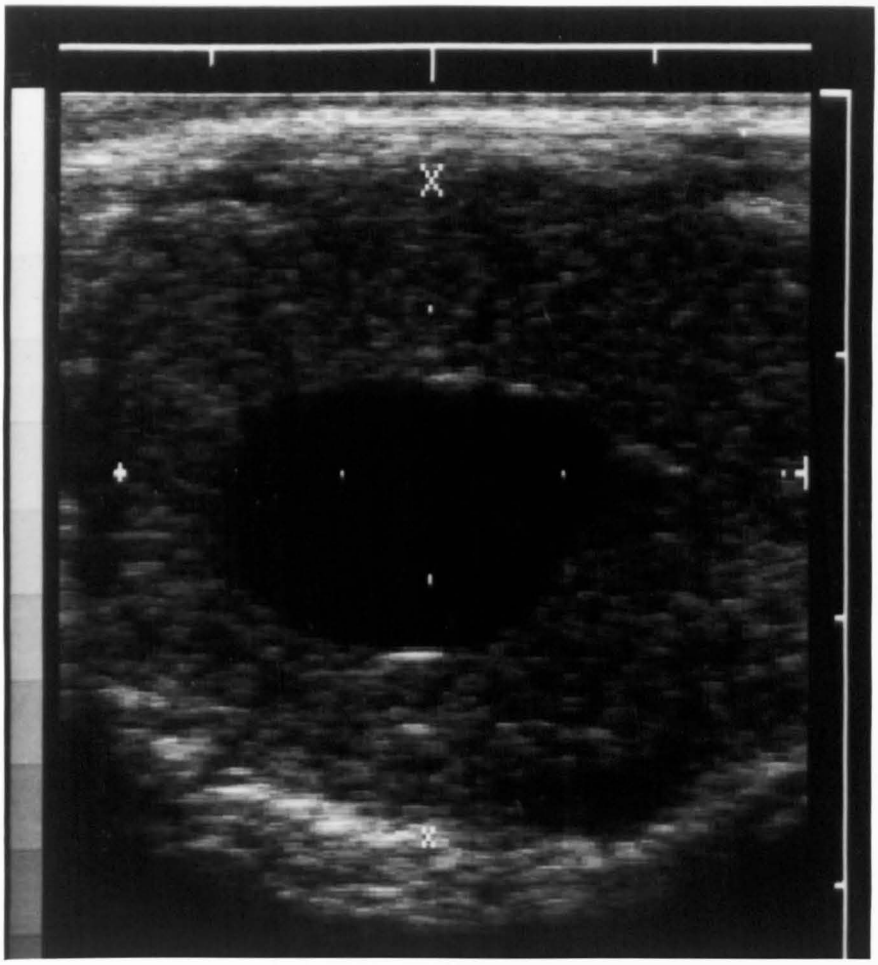


Fig 4.6: Ultrasonogram of CL with cavity

Corpus luteum diameter = 28mm

Cavity diameter = 13mm

Progesterone concentration = 6.04 ng/ml

DISCUSSION

The high correlation (r = 0.85) between CL diameter and progesterone concentration in study 1 was similar to the results of Rajamahendran and Taylor (1990). Although there was a high correlation (r = 0.85) between CL diameter and progesterone profile, our results do not support the conclusion of Kastelic *et al.* (1990) that ultrasonic determination of CL could be a viable alternative to peripheral progesterone determination. In the present study over the last few days of the cycle there was a rapid decline in progesterone concentration, which was not accompanied by a commensurate decline in CL diameter (Ribadu *et al.*, 1992).

Corpora lutea with cavities have been detected previously by ultrasound (Pierson and Ginther, 1984; Reeves *et al.*, 1984; Pieterse, 1989). Progesterone concentration in cow 4 was similar to that in other cows without cavities in the CL confirming the results of Kito *et al.* (1986) and Quirk *et al.* (1986). A CL with a cavity was also noted in the following cycle in this cow.

Both palpation per rectum and ultrasonography had a high sensitivity for identifying mid-cycle CL (85% vs 95.7%) in Study 2. The accuracy of palpation per rectum and ultrasonography for identifying mid-cycle CL is consistent with previous studies (Kähn and Leidl 1986; Pieterse *et al.*, 1990; Archbald *et al.*, 1992; Robertson *et al.*, 1993). The sensitivity of ultrasonography was however higher in the present study (95.7%). The difference may be attributed to differences in types of transducers used and the route of approach (5MHz transducers used per rectum and per vaginum in previous studies). Differences in sample sizes and inter-observer variation may also account for the differences in sensitivities across these studies.

It should be noted that sensitivity, specificity and predictive values depend to a great extent on the quality of the equipment (scanner) and the experience of the operator.

Only one of the three regressing CL observed at dissection (associated with low progesterone) was detected by ultrasonography, while one was incorrectly diagnosed as a mid-cycle CL by palpation per rectum. A similar finding was reported by Pieterse *et al.* (1990) who detected only 4 regressing CL from a total of 11 by ultrasonography and 7 out of 11 by palpation per rectum. The failure to detect the remaining 2 regressing CL by ultrasonography may be related to the slight difference in echogenicity between the regressing CL and the ovarian stroma. Another possible explanation could be failure to image along the plane of the regressing CL.

Only 28% of follicles $\geq$10mm were detected by palpation whereas ultrasonography identified 76%. This result shows that palpation per rectum was a poor method of identifying follicles on the ovaries. Pieterse *et al.* (1990) detected 71% of follicles $\geq$10mm by palpation and 95% by ultrasonography.

In Study 3, palpation correctly diagnosed the presence and type of ovarian cysts in 52% while ultrasound classification was correct in 93%. Misdiagnosis of ovarian cysts may lead to the wrong choice of therapy and/or unnecessary treatment. Using ultrasonography one case of a luteal cyst was wrongly diagnosed as follicular; this may have been related to apparent luteinization of the cyst wall or the presence of an accompanying CL which was not detected using ultrasonography. Nakao *et al.* (1983) correctly classified follicular cysts by palpation (based on milk P_4 and response to GnRH treatment) in 104 (65%) cases; 31 (19%) luteal cysts and 25 (16%) CL with cavities were however wrongly classified as follicular cysts. A lower

proportion (2 of 30 cows (6.7%)) of incorrect diagnosis of ovarian cysts by palpation per rectum was reported by Farin *et al.* (1992) which were revealed by ultrasonography as a CL with cavity and a large follicle (with an accompanying CL).

The progesterone concentration in cows with luteal cysts in the present study was 3.6 $\pm$ 0.49 ng/ml. Progesterone concentration in cows clinically diagnosed as having luteal cysts ranged from 3.0 - 10.4 ng/ml (Leslie and Bosu, 1983) similar to values reported during the luteal phase of the oestrous cycle.

Plasma or milk progesterone is commonly used to augment diagnosis by palpation of ovarian cysts. A low progesterone concentration suggests absence of functional luteal tissue and hence a diagnosis of a follicular cyst, while a moderate or high progesterone concentration indicates the presence of progesterone-secreting luteal tissue and thus supports a diagnosis of a luteal cysts (Kesler and Garverick 1982, Leslie and Bosu 1983, Booth 1988). It should be noted however, that a single progesterone measurement does not confirm the presence of a cyst. If only plasma progesterone determination had been relied upon to confirm the findings of palpation, the 3 cows with large follicles (12 - 14mm) associated with low progesterone (0.46 $\pm$ 0.05 ng/ml) would be wrongly 'confirmed' as having follicular cysts, while the 11 cows with CL (with or without cavities; P_4 6.15 $\pm$ 0.50 ng/ml) would similarly be wrongly 'confirmed' as having luteal cysts.

The identification of corpora lutea (with or without cavities) accounted for the highest misdiagnosis by palpation (79%) in this study. Ultrasonography distinguishes CL (with or without cavities) from luteal cysts by size and echogenic appearance (Kähn and Leidl 1989).

In conclusion, even though there was a high correlation between ultrasound CL diameter and progesterone during most days of the oestrous cycle, congruence was lost during the last few days of the cycle. Both palpation and ultrasonography identified accurately mid-cycle CL : palpation was however less sensitive and poor in detecting follicles and ovarian cysts. Although the high cost of ultrasound equipment currently limits its wider application for bovine fertility work, ultrasonography is a very accurate and reliable method of diagnosis of ovarian cysts and other functional ovarian structures in cattle compared with palpation per rectum.

CHAPTER FIVE

DYNAMICS OF OVARIAN CYSTS MONITORED BY ULTRASONOGRAPHY AND PROGESTERONE PROFILE AND THE EFFECT OF EXOGENOUS OESTRADIOL ON LH RESPONSE

INTRODUCTION

Ovarian cysts in cattle can occur at any stage of lactation (Dinsmore *et al.*, 1987) but commonly during the first 45 to 60 days after calving (Bosu and Peter, 1987; Refsal *et al.*, 1988).

Carroll *et al.* (1990) performed weekly palpation and ultrasonography on 46 cows from day 14 to 100 postpartum and in 11 cows reported spontaneous development of ovarian cysts (3 follicular and 8 luteal). The spontaneous occurrence of ovarian cysts in 4 out of 22 cows was also reported in a study by Savio *et al.* (1990) in which cows were monitored by daily ultrasonography from 5 days after calving until the first ovulation. Plasma progesterone was consistently low (0.1 - 0.3 ng/ml) in 3 of the 4 cows with follicular cysts.

Sawamukai *et al.* (1991) monitored 16 existing cases of ovarian follicular cysts by ultrasonography every 10 days, and milk progesterone profile daily for 20 days before treatment. Neither corpora lutea (CL) nor luteal tissue in the cyst wall were detected by ultrasonography. Ovarian cysts have been reported as dynamic structures which secrete different amounts of progesterone varying with time (Cook *et al.*, 1990; Carroll *et al.*, 1990), and a wide variation in plasma progesterone in cows clinically diagnosed with luteal cysts has been reported: Leslie and Bosu (1983) obtained a range of 3 - 10.4 ng/ml while Carroll *et al.* (1990) reported values of 1-2 ng/ml, well below those for a functional CL.

It has been reported that the feedback regulation of oestradiol on the hypothalamic-pituitary axis is altered in cystic ovarian degeneration (Zaied *et al.*, 1981; Eyestone and Ax, 1984; Dobson and Alam, 1987; De Silva and Reeves, 1988). Nanda *et al.* (1991) reported failure of a luteinizing hormone surge in response to oestradiol in 50% of cows with cystic ovarian disease. It is not clear why some cows with ovarian cysts respond to oestradiol while others do not. There is considerable variation in tonic pulsatile release of gonadotrophins in cows with ovarian cysts, even among individuals with similar sex steroid profiles (Refsal *et al.*, 1988).

The concentration of LH in cows with ovarian cysts is generally reported to be higher than in normal cows and inversely correlated to concentration of plasma progesterone (Youngquist, 1986). It is well known that high progesterone concentration inhibits the positive feedback effects of oestradiol in cows (Hansel and Convey, 1983; Peters, 1986) and ewes (Currie *et al.*, 1993).

Ultrasonography has enabled reliable monitoring and characterization of ovarian follicular growth pattern during the oestrous cycle (Pierson and Ginther, 1984; Savio *et al.*, 1988; Sirios and Fortune, 1988b; Knopft *et al.*, 1989), but there is a paucity of detailed information on ultrasound and progesterone monitoring of ovarian cysts and of follicles associated with cysts in cattle. The objectives of the present studies were therefore to:-

(1) examine by ultrasonography and progesterone measurement the initial occurrence of ovarian cysts after calving and the response to exogenous oestradiol.

(2) monitor by ultrasonography and progesterone clinical cases of ovarian cysts, follicular dynamics in the presence of cysts and the response to exogenous oestradiol.

MATERIAL AND METHODS

Animals

All cows used in this study were located on farms of the Liverpool University Farm Animal Practice in Wirral, North-West England. A total of 34 cows (18 for the early postpartum study and 16 clinical) cases were used.

Ovarian cysts were diagnosed by palpation per rectum (day 1), ultrasonography and plasma progesterone. Palpation diagnosis was based on reproductive history (irregular oestrus, or anoestrus) and palpation of a large fluid-filled follicular structure(s) on an ovary in the absence of a CL (Seguin, 1980). By ultrasonography, a follicular cyst was defined as a uniformly nonechogenic ovarian structure $\geq$25mm in diameter with a wall $\leq$3mm thick (Chapt. 4: Fig 4.3), while a luteal cyst was a nonechogenic ovarian structure with grey patches within the antrum or along the inner cyst wall with a wall thickness $>$3mm (Chapt. 4: Fig 4.4). Plasma progesterone $<$0.9 ng/ml and $>$0.9 ng/ml was also a criterion for follicular and luteal cysts, respectively. Ultrasound and blood sampling was done in the first study from day 7 and then weekly until day 42-49 postpartum. In the second study, cows in which cysts developed could only be scanned and blood sampled every other day from day of diagnosis until day 12, since they were on commercial farms.

Ultrasonography

Ultrasound examination was performed using an ultrasound scanner (Aloka, BCF Technology, Livingstone, U.K.) equipped with a 7.5 MHz rectal transducer (Aloka, UST 55111, Japan). Maximum diameter of follicles $\geq$5mm, corpora lutea and inner and outer diameter of cysts were measured after freezing the image on the screen. Prints of representative images were obtained using an on-line thermal printer (Sony, Video Graphic UP-850, Japan).

Treatment

On day 11, all cows with luteal cysts were injected intramuscularly with 500 μg of cloprostenol (2 ml Estrumate, Coopers, Pitman-Moore, U.K.). On day 12 after cyst diagnosis (24 h after cloprostenol in luteal cases) all cows received intramuscularly 1 mg of oestradiol benzoate (Intervet, Ltd, U.K.) dissolved in 2 ml arachis oil. A group of 6 cyclic control cows with a functional CL were injected with cloprostenol and oestradiol benzoate 24 h later.

Blood sampling

A 10 ml blood sample was obtained after each examination by tail venepuncture into a heparinised Vacutainer tube (Becton, Dickinson Vacutainer systems, Runcorn, U.K.). Further blood samples were obtained in all cows with cysts on day 12, before 18 and 24 h after oestradiol injection. Control cows were also bled at the time of oestradiol injection and at 18 and 24 h after oestradiol. Blood was centrifuged at 1000 g and stored at -20°C until analysis for progesterone and LH.

Hormonal analysis

Plasma progesterone was measured by radioimmunoassay (Kanchev *et al.*, 1976). Plasma LH was measured by a verified double antibody radioimmunoassay (Alam and Dobson, 1986).

Statistical analysis

An LH surge in response to oestradiol was confirmed when the LH concentration increased to >10 ng/ml in samples collected 18 and, or, 24 h after oestradiol. Luteal tissue volume in luteal cysts and cyst wall volume of follicular cysts was calculated by subtracting the cyst's cavity volume from total cyst volume using the formula: $V = \pi d^3/6$, where V = volume of cyst or cyst cavity, $\pi = 3.14$, d = diameter of cyst or cyst cavity (Munday and Farrar, 1979). Correlation between (i) progesterone increase and ultrasound detection of CL in early postpartum cows, (ii) progesterone and luteal tissue volume of luteal cysts and (iii) progesterone and follicular wall volume of follicular cysts were examined using Spearman's correlation. For all cows with cysts over the monitoring period the mean number of all follicles $\geq$5mm in the ipsilateral ovary was compared to those in contralateral ovary to the cyst using Student's *t* test.

RESULTS

Study 1

Of 18 cows monitored in Study 1 during the early postpartum period, 16 cows started ovarian cyclicity by day 14-28 postpartum. A CL had been observed during ultrasonography in 5 cows by day 14, in 12 cows by day 21 and in 16 cows by day 28. There was complete agreement ($r=1$, $p<0.05$) between ultrasound

detection of a CL and progesterone increases in all the 16 cows that did not develop ovarian cysts. In 2 of these 16 cows, however, CL (of similar appearance and location) and high progesterone persisted for 28-35 days. The two cows remaining had large follicles 20-22mm on day 35 and had spontaneously developed an ovarian luteal cyst by day 42.

Study 2

Cysts were diagnosed 80.8 $\pm$ 9.4 days postpartum in the 16 cows in Study 2. Mean external diameter on day of diagnosis was 40.3 $\pm$ 3.7 mm for follicular, and 36.4 $\pm$ 1.3 mm for luteal cysts. The difference in size between the two types of cyst on day of diagnosis was not significant ($P>0.05$). A total of 72% occurred in the right ovary while 28% were in the left ovary. None was bilateral. Normal diameter of mid-luteal phase CL range between 20 - 26mm (Fig 4.1).

The relationship between follicular cyst wall volume or luteal wall volume and plasma progesterone is presented in Figs 5.1 and 5.2 while Figs 5.3 and 5.4 represent mean $\pm$ S.E.M. diameter of follicles associated with follicular and luteal cysts, respectively. Changes in individual cysts and follicles with time and LH measurement after oestradiol is provided as appendix IB, IC and ID respectively.

The wall volume in follicular cysts and the volume of luteal tissue in luteal cysts remained fairly constant. The luteal tissue volume decreased, but not significantly ($P>0.05$) 24 h after cloprostenol compared to that during days 1 - 11 (Fig 5.2) and luteal tissue was still visible on ultrasound though less homogenous. There was no correlation between cyst wall volume of follicular cysts and plasma progesterone ($r=0.04$, $p>0.05$). Although a correlation of 0.54 was obtained

between luteal tissue volume and plasma progesterone ($p > 0.05$), the association was not significant.

Progesterone concentration in all cows with follicular cysts remained consistently low < 0.9 ng/ml. In cows with luteal cysts, the progesterone concentration ranged between 2.78 ± 0.83 ng/ml and 4.86 ± 0.92 ng/ml, but 24 h after prostaglandin progesterone values decreased from 2.78 ± 0.83 to 0.63 ± 0.15 ng/ml.

There were significantly ($P < 0.05$) more follicles in the contralateral ovary (11.4 ± 0.9 vs 2.0 ± 0.6) than in the ovary bearing the cyst (irrespective of cyst type). A wave pattern of follicular growth in the presence of cysts was observed in the ovaries of 16 of 18 cows (5 follicular; 11 luteal). The size (external and internal diameter) increased with time in only 1 follicular cyst. It remained fairly constant in 14 cases (5 follicular; 9 luteal) and decreased in 3 cases (1 follicular and 2 luteal). Overall, mean cyst diameter (external and internal) on day 11 was also not different ($P > 0.05$) compared to days 1, 3 and 5. In one cow with follicular cyst, another cyst developed on the same ovary on day 7 after initial diagnosis of the first cyst. Sizes of F1 (largest) and F2 (second largest) follicles on days 9, 11 and 12 were not significantly greater ($P > 0.05$) than days 1, 3, 5 and 7 in both follicular and luteal cysts. Mean follicle sizes on day 1 in both types of cyst was also not different ($P > 0.05$) (Fig 5.3 and 5.4).

Five of six (83%) control cows had an LH surge at 18 and, or, 24 h after oestradiol administration. One of 7 (14.3%) follicular cysts and 3 of 11 (27.3%) luteal cysts responded with a surge of LH (> 10 ng/ml) to oestradiol treatment. Plasma progesterone was low (< 0.9 ng/ml) in all control cows and cows with cysts

at the time of oestradiol treatment. In addition, one cow with follicular cyst and one with luteal cyst had high concentration of LH; 14.7 and 19.5 ng/ml respectively, at the time of oestradiol treatment.

Fig 5.1: Dynamics of follicular cysts (n=7)

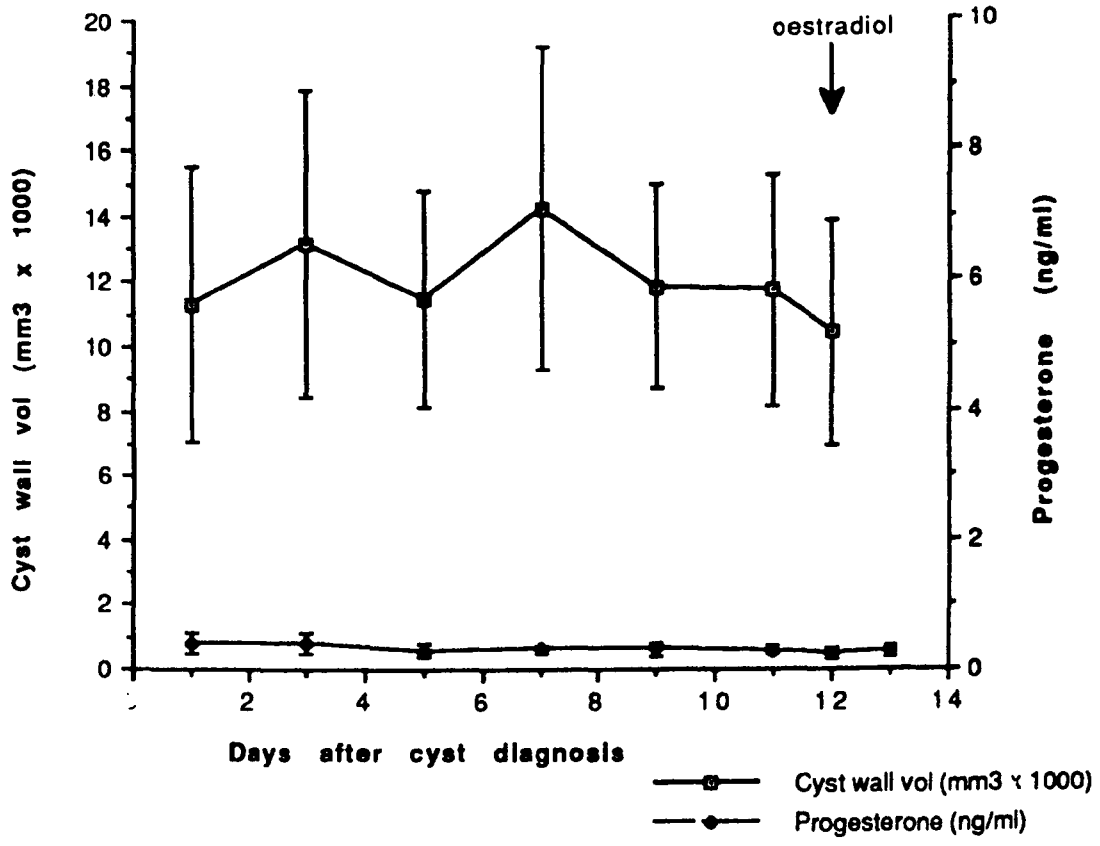


Fig 5.2: Dynamics of luteal cysts (n=11)

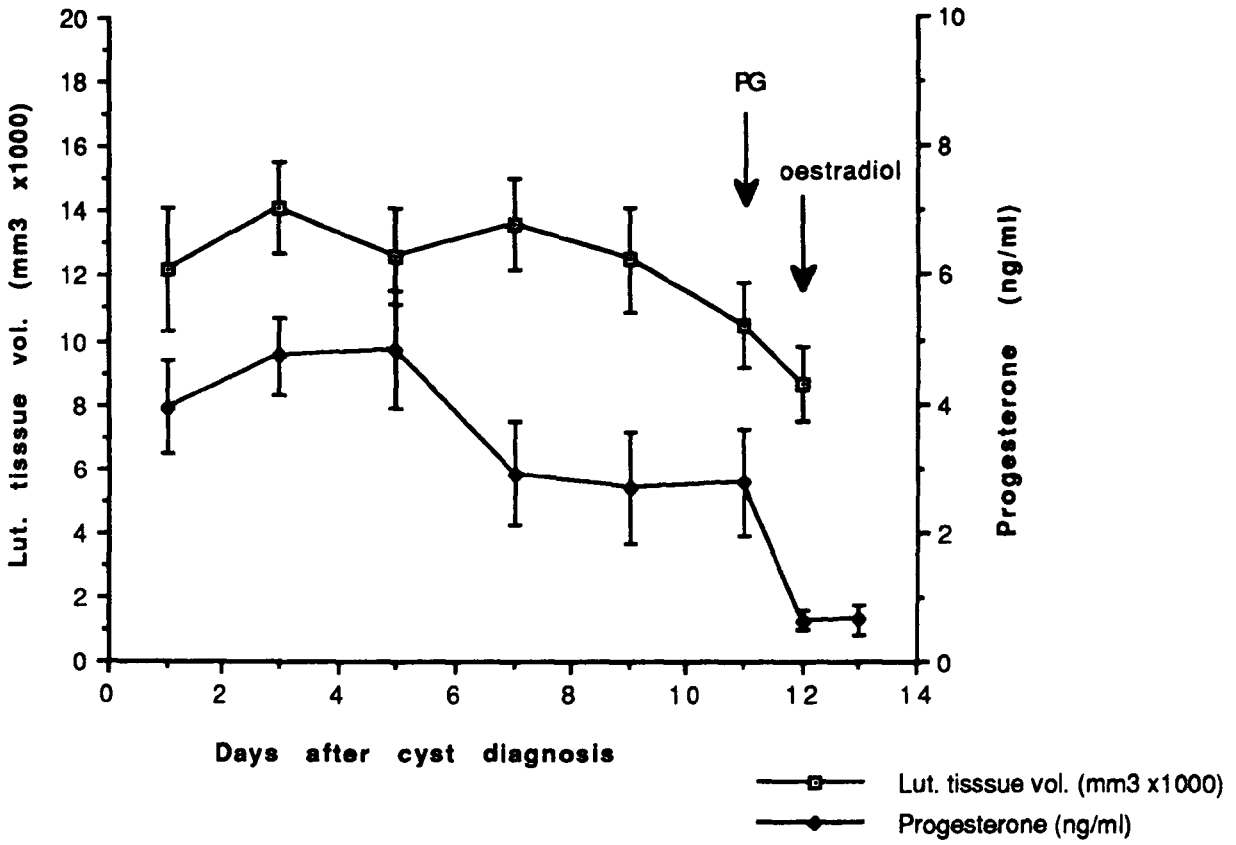


Fig. 5.3: Dynamics of follicles in the presence of follicular cyst (n=7 cows)

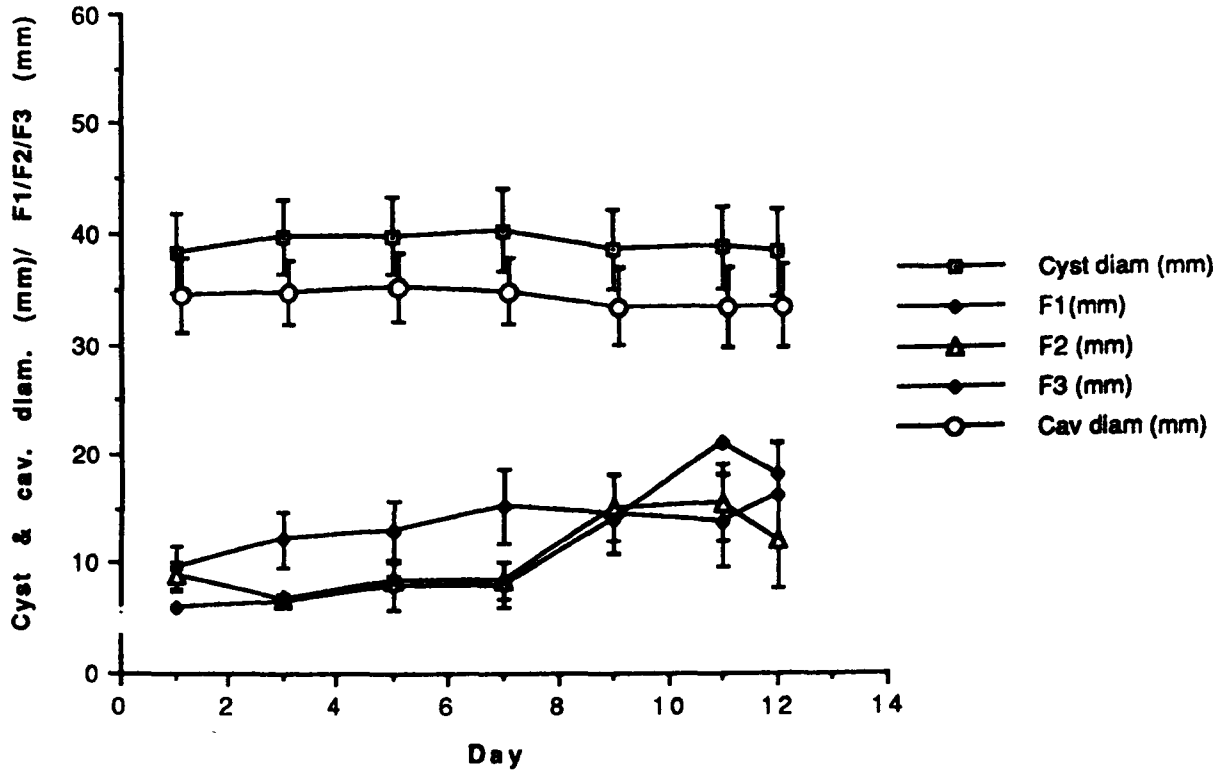
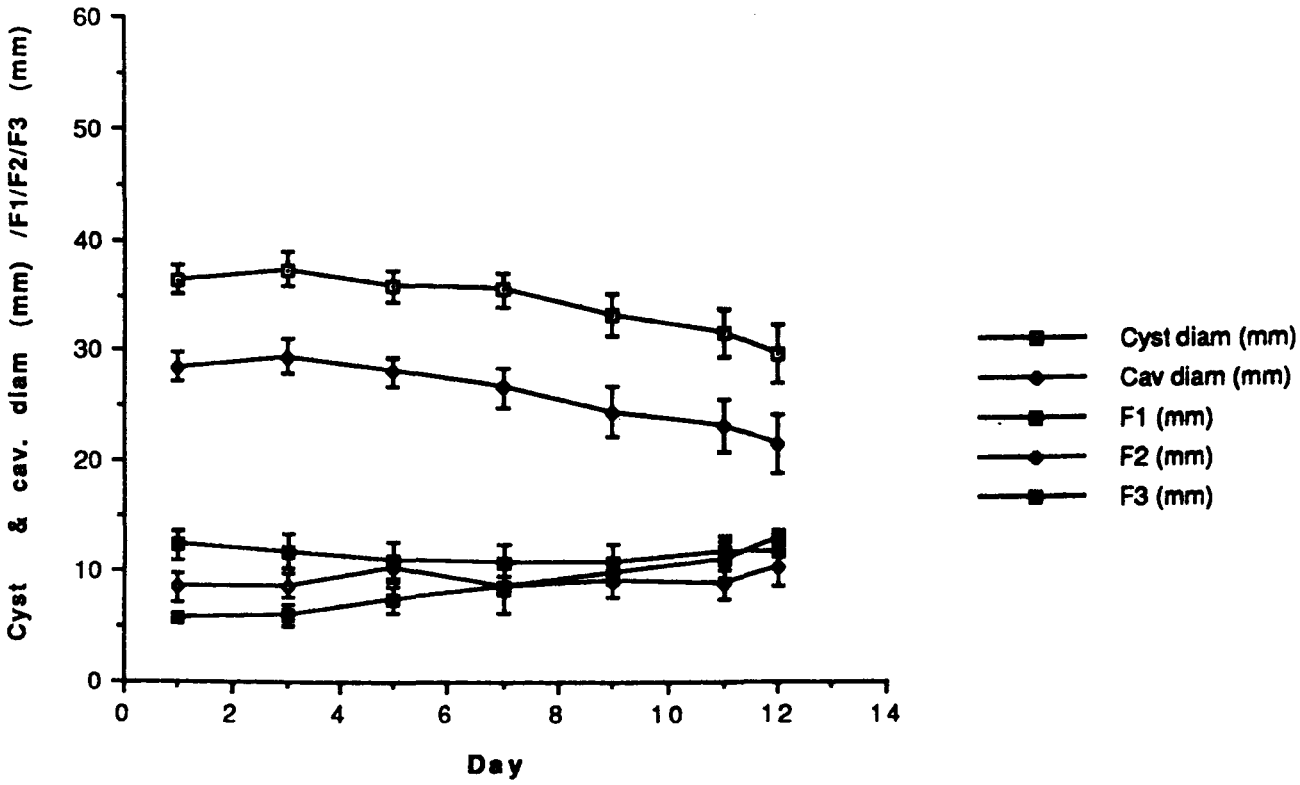


Fig 5.4: Dynamics of follicles in the presence of luteal cysts (n=11 cows)



DISCUSSION

In Study 1 (early postpartum) 16 of 18 cows did not develop ovarian cysts. Of these, 5/16 (31%) had an increase in progesterone values by 14 days postpartum, 12/16 (75%) by day 21, and 16/16 (100%) by day 28. There was complete agreement between ultrasound detection of CL and a rise in plasma progesterone. Lamming and Bulman (1976) reported significant increases in milk progesterone concentration in 50% of cows before day 20 postpartum and ovarian activity had commenced in 93% by day 40 postpartum. Persistent CL accompanied by high progesterone was noted in 2 cows in this study. Corpora lutea of similar ultrasound appearance and size were located in the same ovaries on consecutive weekly examinations, and the CL were therefore most unlikely to be new CL as a result of oestrus in between weekly observations. Lamming and Bulman (1976) similarly observed persistently high milk progesterone for at least 30 days in 2% of cows studied.

The number of cows that developed ovarian cysts during Study 1 (11%) in the present study was lower than the reported previously; 18.2% and 23.9% by Savio *et al.* (1990) and Carroll *et al.* (1990) respectively. The difference between these studies may relate to small sample sizes and possibly differences in breed, age, lactation numbers and husbandry conditions.

During the 12 day observation period, luteal tissue was not observed by ultrasonography in all the follicular cysts nor was progesterone elevated. Savio *et al.* (1990) reported luteinization of 1 of 4 follicular cysts whereas Sawamukai *et al.* (1991) concurred with the present study in all 16 cases of follicular cysts observed over a 20 day period.

During Study 1, two cows with large follicles (20-22mm) spontaneously developed luteal cysts a week later. In seven cows with follicular cysts in Study 2 the cysts failed to luteinize over the 12 day observation period. It is unknown why some cysts spontaneously luteinize while others remain follicular (with regression and or development of new follicular cyst). Since the cows belonged to commercial farms, all cows in this study had to be treated 2 weeks after initial diagnosis. It is possible that luteinization of cysts may be observed if cows with cysts were monitored much longer.

A correlation of 0.54 was obtained between luteal tissue volume and plasma progesterone. The low correlation may be attributed to high variability in plasma progesterone concentration and in luteal tissue volume in cows with luteal cysts. The absence of a correlation between follicular cyst wall volume and plasma progesterone is not surprising because follicular cysts tended to be larger and the cyst wall volume substantial, but as a result of lack of luteinization progesterone remained low (<0.9 ng/ml). Plasma progesterone concentration in luteal cases decreased from 2.78 ± 0.83 ng/ml to 0.63 ± 0.15 ng/ml 24 h after cloprostenol injection. Luteal tissue was, however, visible by ultrasonography (though slightly less homogenous). This re-emphasises the point that morphological presence is not always equated with physiological function (Ribadu *et al.*, 1992).

The wall thickness in both types of cyst remained fairly constant. There was however, an increase in both external and internal cyst diameter in 1 cow with follicular cyst, decrease in 3 cows and a new cyst formation in 1 cow. These changes with time may relate to the dynamic nature of cysts (Cook *et al.*, 1990; Mujuni *et al.*, 1993). Even though follicular cysts seemed larger than luteal cysts,

the difference was not significant. Leslie and Bosu (1983) reported that follicular cysts were significantly larger than luteal cysts. A higher proportion of cysts (72%) in this study occurred on the right ovary. Bierchwal *et al.* (1975) also made a similar observation.

The observation in this study shows for the first time that there were significantly ($P < 0.05$) more follicles in the contralateral ovary than in the ipsilateral ovary (irrespective of cyst type) suggesting that the cyst may inhibit the development and maturation of other follicles on that ovary.

The hypothalamic-pituitary feedback of control and cystic cows was evaluated in this study by measuring the release of plasma LH by exogenous oestradiol. A surge of LH was obtained in 5/6 (83%) control cows. The failure of 1 control cow to respond could not be explained, because progesterone at the time of oestradiol injection was <0.9 ng/ml. Zaied *et al.* (1981) reported that responsiveness to oestradiol is regained 2 to 4 weeks postpartum in most cows. All cows in this study were >35 days postpartum.

Overall, in 4/18 (22%) of cows an LH response was obtained. This is higher than reported by Dobson and Alam (1987) and Refsal *et al.* (1988) but lower than the 50% response from this laboratory recently (Nanda *et al.*, 1991). The difference may be attributed to difference in number of and types of cases. One follicular cyst and 1 luteal cyst had spontaneous surges of LH at the time of oestradiol injection. Those cows may be considered as potentially capable of producing an LH surge in response to oestradiol. It should be noted that apart from the 2 luteal cysts observed during postpartum monitoring, the remaining 16 ovarian cyst cases were already established cases at the time of diagnosis. It was not possible to determine the time

of initial occurrence. It may be that the response of cows with cysts to exogenous oestradiol varies with time after cyst formation.

Nanda *et al.* (1991) suggested that progesterone treatment of ovarian cysts could heal the prevailing endocrine lesion and improve the LH surge response to oestradiol. It was further argued that failure of 53% of luteal cysts in that study to respond to oestradiol may have been due to short term (less than 7 days) exposure to progesterone. In the present study, 73% of luteal cysts failed to respond to oestradiol even though functional luteal tissue was present in all for at least 11 days before prostaglandin. It may be that a factor in the persistence and perhaps initiation of cysts is refractoriness to the positive feedback effect of oestradiol on gonadotrophin release (Refsal *et al.*, 1988).

In conclusion, spontaneous luteinization of follicular cysts was not observed in this study. Response of cows with cysts to exogenous oestradiol was low. The presence of a cyst seemed to be detrimental to the development and maturation of follicles in the same ovary.

CHAPTER SIX

ULTRASOUND AND PROGESTERONE MONITORING OF OVARIAN FOLLICULAR CYSTS IN COWS TREATED WITH GnRH

INTRODUCTION

Cystic ovarian degeneration is a major reproductive disorder of cattle. It is characterised by the presence of one or more follicular structures larger than 25mm in diameter for 10 days or more in the absence of a corpus luteum (Seguin, 1980).

GnRH (a decapeptide) is commonly used for the treatment of ovarian cysts. The advantages of GnRH are its low price, lack of refractoriness due to antibody formation and the fact that anaphylaxis following GnRH treatment has not been reported in cows (Bosu, 1982; Youngquist, 1986; Drost and Thatcher, 1992).

Luteal tissue development following GnRH treatment in cattle may be monitored indirectly by measurement of progesterone concentration in plasma or milk. Garverick *et al.* (1976), using palpation per rectum described cyst luteinization that was characterised by increased firmness and reduced cyst size in cows that responded to GnRH or human chorionic gonadotrophin (hCG) treatment. Nakao *et al.* (1983) found a correlation of only 30% between palpation per rectum 10 days after GnRH treatment in assessing luteinization of follicular cyst with milk progesterone analysis. Incorrect assessment of luteinization of follicular cysts by palpation after treatment has been a problem (Whitmore *et al.*, 1979; Nakao and Kawata, 1980). With the introduction of ultrasonography, it may be possible to monitor objectively the development of luteal tissue in order to evaluate effectiveness of treatment of ovarian cysts.

Plasma progesterone concentration is elevated 5-9 days following GnRH treatment in most cows that respond positively (Cantley *et al.*, 1975; Garverick *et al.*, 1976; Seguin *et al.*, 1976; Kesler *et al.*, 1978) as a result of luteinization of the cyst or of other follicles with or without ovulation (Kesler *et al.*, 1977b; Youngquist, 1986).

Although endocrine changes following GnRH treatment of ovarian cysts in cattle have been described (Cantley *et al.*, 1975; Garverick *et al.*, 1976; Seguin *et al.*, 1976; Kesler *et al.*, 1978; Nanda *et al.*, 1988), information on ultrasound changes following treatment is scarce. The aim of the present study was therefore to monitor ovarian ultrasound changes and correlate with plasma progesterone in cows with follicular cysts that had been treated with GnRH.

MATERIAL AND METHODS

Five lactating Friesian cows (799, 72, 145, 603, and 457) on 3 dairy farms were diagnosed as having follicular cysts (by palpation per rectum, ultrasonography and progesterone determination). The diagnosis by palpation per rectum was based on the finding of a single or multiple follicular structures of at least 25mm in diameter on one or both ovaries in the absence of a corpus luteum. By ultrasonography a follicular cyst was defined as a uniformly nonechogenic ovarian structure $\geq$ 25mm in diameter with a wall $\leq$3mm. A plasma progesterone concentration of $<$0.9 ng/ml was used to further classify the cysts as follicular.

Ten days after initial diagnosis (day -10), cows were treated (day 0) with 500 ug synthetic GnRH (gonadorelin, 5 ml Fertagyl; Intervet Labs Ltd, U.K.) and

ovarian ultrasonography and blood sampling was carried out weekly until day 35 after treatment.

Ultrasound examination was performed using an ultrasound scanner (SSD 210 DXII, Aloka, BCF Technology, Livingstone, U.K.) equipped with a 7.5 MHz rectal transducer (Aloka UST 55111, Japan). Representative images were obtained at each examination using an on-line thermal printer (Sony, Video Graphic UP 850, Japan).

A blood sample was obtained by tail venepuncture after each examination. Blood was centrifuged at 1000 g and stored at -20°C until analysis for progesterone. Plasma progesterone was measured using a validated radioimmunoassay (Kanchev *et al.*, 1976).

Therapeutic success was confirmed by the development of CL and/or luteinization of cysts (which was characterised by increased cyst wall thickness with granular echogenic appearance as visualised by ultrasound) and increased plasma progesterone concentration. Therapeutic failure was defined as cases in which cysts remained unchanged or new cysts develop without luteal development and the cow fails to cycle normally.

RESULTS

Ovarian ultrasound findings during the weekly examinations are summarised in Table 6.1. The relationship between cyst size and progesterone concentration is given in Figs. 6.1 and 6.2. An example of an ultrasonogram of a follicular cyst on the day of diagnosis (day 0) is given in chapter 4; Fig 4.3, while Figs 6.3-6.5 are

ultrasonograms of a luteinized follicular cyst (day 7), luteinized follicular cyst (day 14) and 2 GnRH - induced corpora lutea (day 7).

Prior to treatment three cows showed recurrent oestrous behaviour and two anoestrus. All five cows responded positively to GnRH treatment. By day 7 after treatment, luteinization of cysts (Figs 6.3 & 6.4) was noted during ultrasonography in 2 cows while GnRH-induced CL were observed in the remaining 3 cows (Fig 6.5; Table 6.1).

Changes noted during weekly ultrasonography after treatment included clouding of the uniformly nonechogenic antrum of cysts, luteinization of cyst walls, reduction in size or resolution of the cyst, and, or, development of 1-4 corpora lutea in the ovary bearing the cyst and/or in the contra-lateral ovary. All 5 cows were in oestrus 18.80 $\pm$ 1.74 days after treatment.

Mean ($\pm$ S.E.M.) plasma progesterone concentration on day -10 and day 0 were 0.2 $\pm$ 0.13 ng/ml and 0.72 $\pm$ 0.27 ng/ml respectively. Progesterone concentration was elevated (5.8 $\pm$ 0.67 ng/ml) in all cows by day 7.

DISCUSSION

The visualisation by ultrasonography of cyst luteinization and, or, the presence of CL in all cows by day 7 after GnRH shows that in responding cases, evidence for therapeutic success could be confirmed quickly. All five cases in this study responded positively by either cyst luteinization and, or, CL formation. Failure to detect luteinization of follicular cysts accurately after GnRH by palpation per rectum has led to unnecessary treatment in many cows (Nakao *et al.*, 1983). Ultrasonography could prevent this problem. It has been reported that GnRH has

about 80% success rate in treating ovarian cysts in cattle (Kesler and Garverick, 1982).

In this study, follicular cysts persisted (though in reduced size) for 3-4 weeks after GnRH in the presence of a CL; such cysts may not have any functional significance. Cow 457 had only one follicular cyst and 3 large follicles (15 - 20mm) at initial diagnosis (day -10), but, 3 follicular cysts were visualised during ultrasonography at the time of treatment (day 0). This observation supports the proposition that ovarian follicular cysts are dynamic structures (Cook *et al.*, 1990).

Exogenous GnRH acts on the pituitary gland to cause release of endogenous LH (Garverick *et al.*, 1976; Youngquist, 1986). Response of cows with ovarian cysts appears to vary with the dose of GnRH administered (Youngquist, 1986). If sufficient GnRH is given and mature follicles are present, ovulation is induced within 24 - 30h (Lee *et al.*, 1983). The integrity of cyst granulosa and theca cells may also be an important factor determining the response to treatment (Bierschwal *et al.*, 1980).

The observation of an increase in progesterone by day 7 after treatment is consistent with previous studies (Cantley *et al.*, 1975; Garverick *et al.*, 1976; Seguin *et al.*, 1976; Kesler *et al.*, 1978). Progesterone concentration remained low <0.9 ng/ml in all cows from day -10 to day 0. Cantley *et al.*, (1975) found higher mean pre-treatment concentration of progesterone in cows that responded to GnRH than in those that did not respond.

It is evident that ultrasonography and progesterone measurement are useful tools for evaluating the response of follicular cysts to GnRH in cows.

Fig 6.1: Dynamics of follicular cysts that initially responded by cyst wall luteinization (n=2)

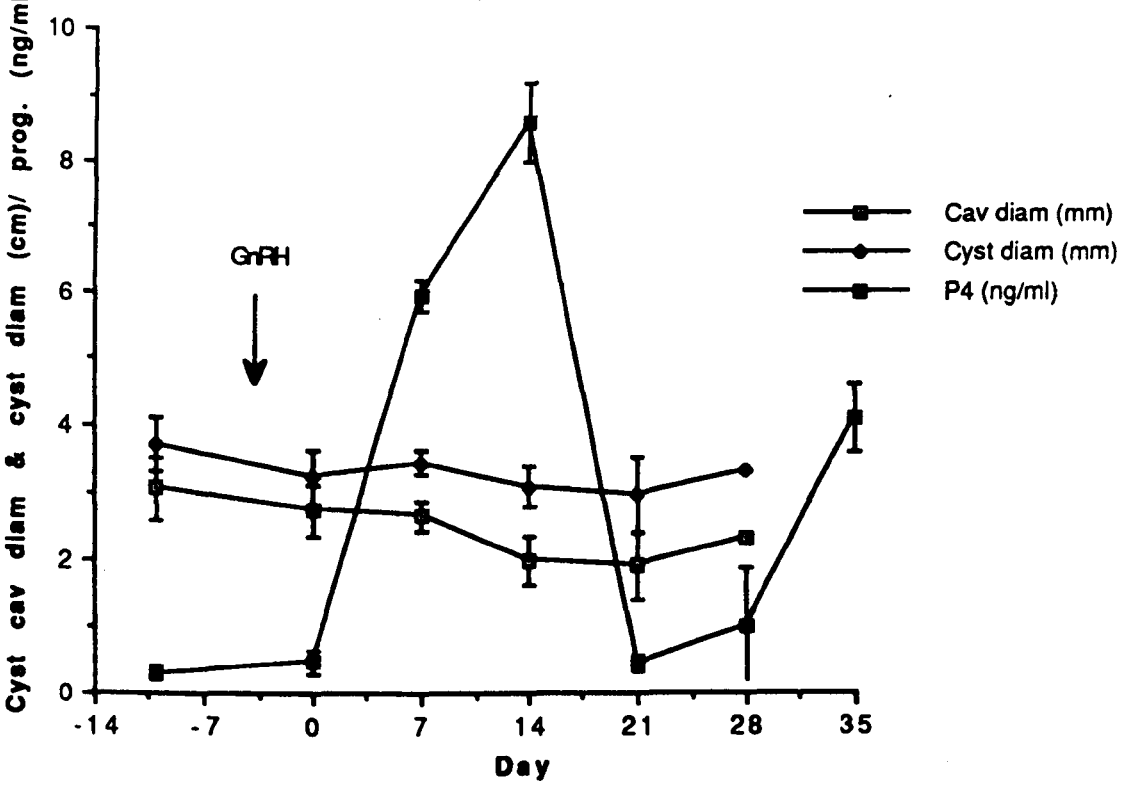
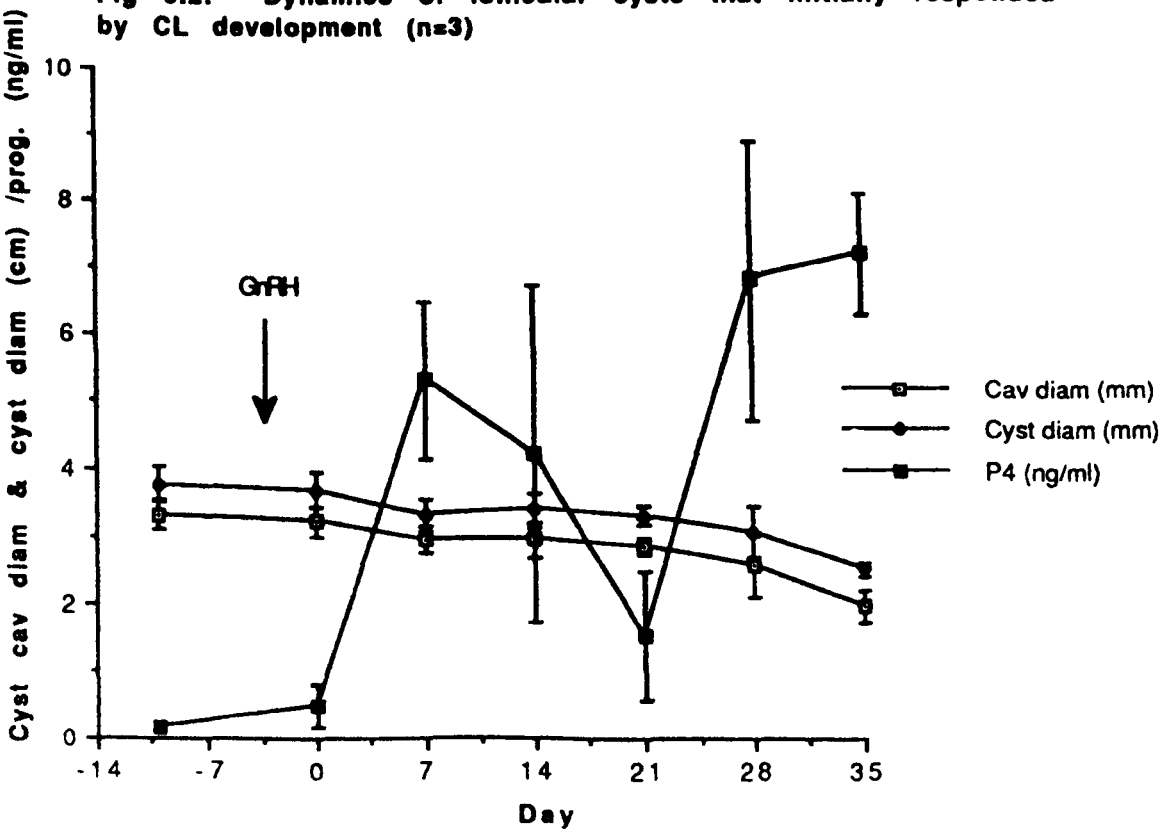


Fig 6.2: Dynamics of follicular cysts that initially responded by CL development (n=3)



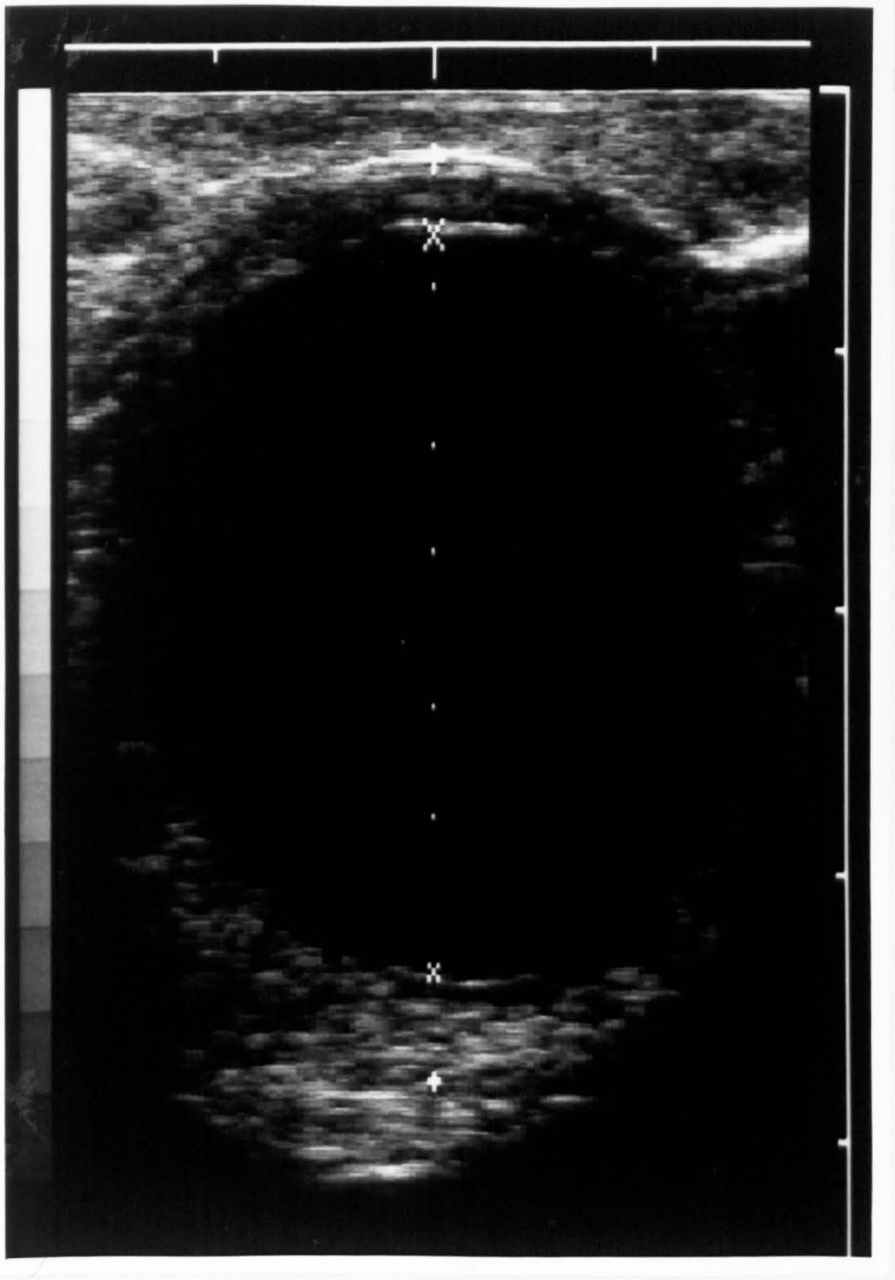


Fig 6.3: Ultrasonogram of a luteinized follicular cyst (7 days after treatment)

diameter of cyst cavity = 28mm

diameter of cyst = 35mm

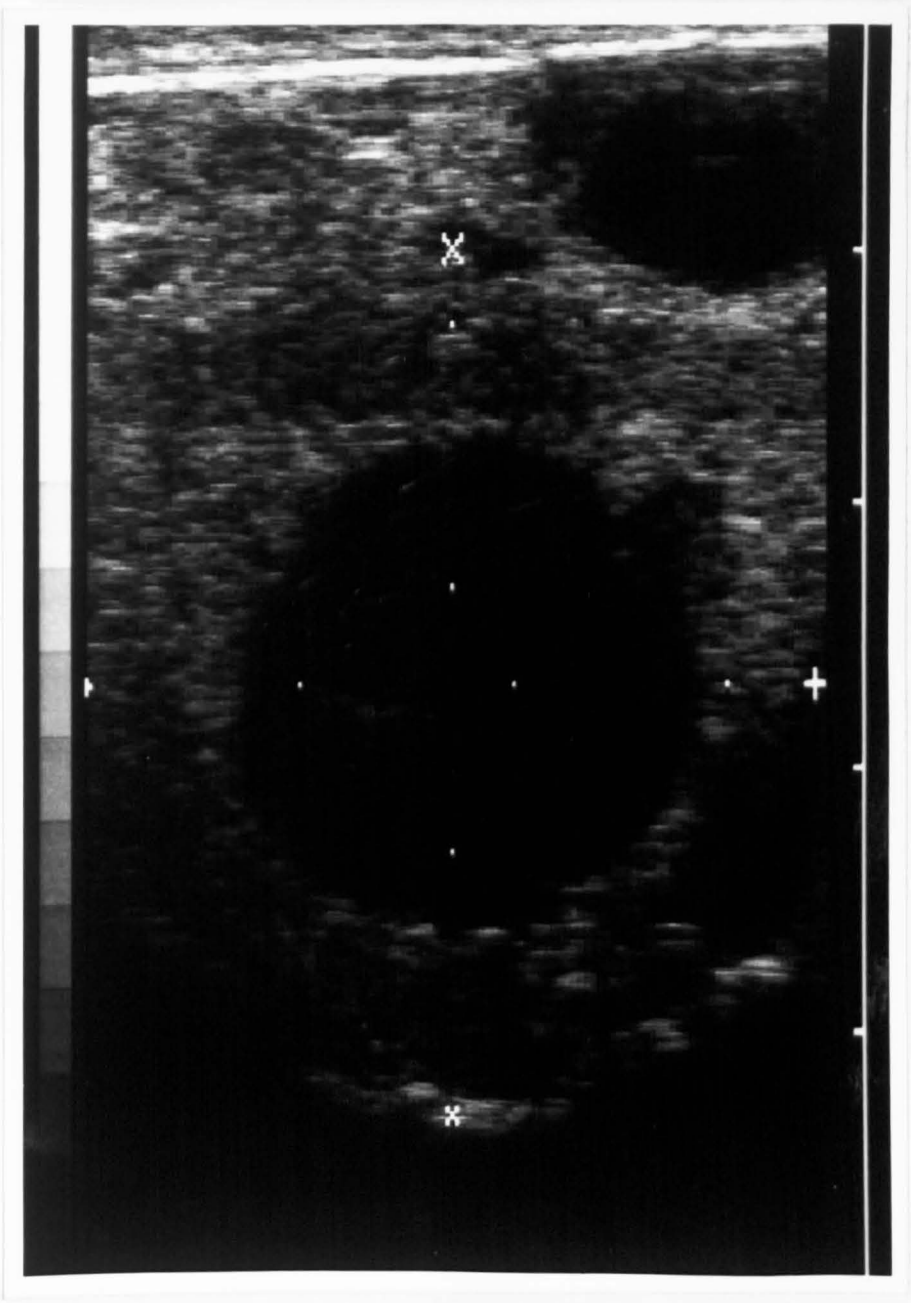


Fig 6.4: Ultrasonogram of a luteinized follicular cyst (14 days after treatment)

diameter of cyst cavity = 18mm

diameter of cyst = 33mm

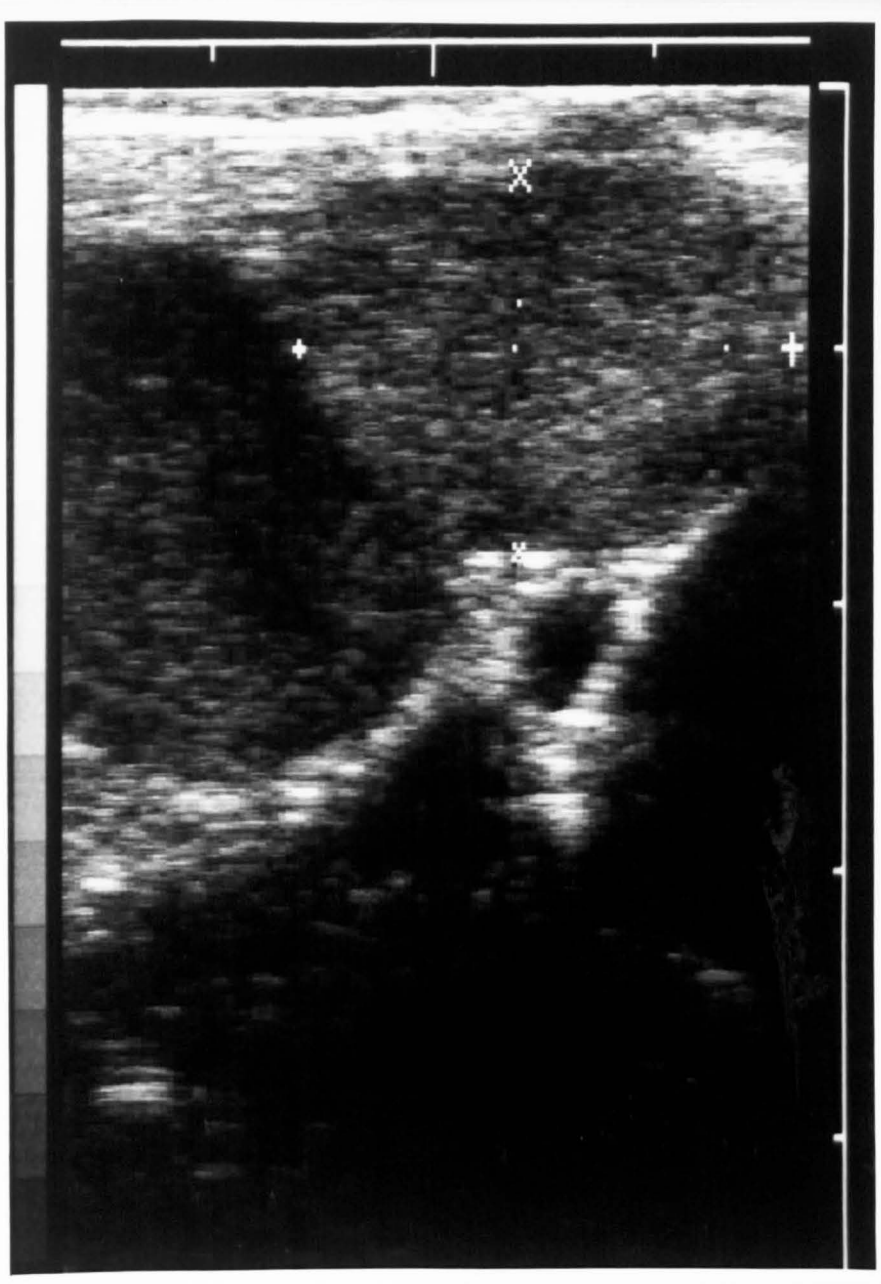


Fig 6.5: Ultrasonogram of 2 GnRH - induced CL (7 days after treatment)

CL diameter = 20mm

Table 6.1. Ultrasound findings on both ovaries before and after treatment of follicular cysts in five cows treated with GnRH

Cow	Day -10	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35
799	FC + 2F	FC + 3F	FC + 4CL	FC + rCL	FC + CL	FC + CL	rFC + CL
72	FC + F	FC + 3F	Lut.FC +Lut.F	Lut.FC + Lut. F + CL	Lut.FC + Lut. F	Lut.FC + CL + F	F + CL
145	2FC + F	2FC + F	2FC + F + CL	2FC + CL	2FC + F + rCL	FC + 4F + CL	3F + CL
603	2FC + F	2FC + 2F	2FC + CL	2FC + CL	2FC + F	FC + F + CL	rFC + F + CL
457	FC + 2F	3FC	3Lut.FC + F	3Lut.FC	rLut.FC +F	CL	F + CL

FC = Follicular cyst

F = Follicle $\geq$ 10mm

rFC = Regressing follicular structure <25mm

Lut.FC = Luteinized follicular cyst (cyst wall >3mm thick)

Lut.F = Luteinized follicle (large follicle with a thick wall >3mm)

rLut.FC = regressing luteinized follicular cyst

CL = Corpus luteum

rCL = regressing CL

CHAPTER SEVEN

ULTRASOUND AND HORMONE PROFILE OF EXPERIMENTALLY INDUCED OVARIAN CYSTS IN CATTLE

INTRODUCTION

There are several limitations on studies of spontaneous cases of ovarian cysts. As the cases are already established, it is impossible to know for how long the cystic structure(s) have existed before diagnosis. In addition, the circumstances affecting ovarian function at the time of diagnosis may be different to the prevailing conditions at the time of study. It is also difficult to follow clinical cases (once diagnosed) for a long period without treatment as clients request treatment as soon as possible. It may, therefore, be helpful to induce ovarian cysts experimentally in order to monitor their development closely. It is not known whether the morphological and endocrine differences seen in clinically diagnosed ovarian cysts represent stages of a single or multiple pathophysiological mechanism(s) (Refsal *et al.*, 1987). Comparison with induced cysts may clarify the position.

Ovarian cysts in cattle have been induced by either a single intramuscular injection of oestradiol valerate (5 mg) on day 16 of the oestrous cycle or 100 ml of antiserum to bovine luteinising hormone (LH) before the onset of oestrus (Wiltbank, 1966; Nadaraja and Hansel, 1976). Injection of increasing doses of testosterone during the follicular phase of the oestrous cycle resulted in formation of ovarian cysts in 5 cows (Fathala *et al.*, 1978) whereas Cook *et al.* (1990) experimentally induced ovarian cysts in cows by repeated injections of high doses of progesterone and oestradiol.

Stress has been implicated in the aetiology of spontaneously occurring ovarian cysts in cattle. The preovulatory LH surge is sensitive to the inhibitory effects of exogenous adrenocorticotrophic hormone (ACTH) (Stoebel and Moberg, 1982 Dobson *et al.*, 1988). Inhibition of the preovulatory LH surge by ACTH treatment resulted in the development of ovarian cysts in cows (Liptrap and McNally, 1976; Refsal *et al.*, 1987) and ewes (Cooke and Benhaj, 1989; Doney *et al.*, 1976).

Savio *et al.* (1990) monitored spontaneous cyst formation in 4 cows and from that data it can be seen that there are two distinct phases of cyst development; a functional phase lasting up to 20 days during which high oestradiol secretion occurred, followed by a structural phase of similar duration with basal oestradiol concentration. Sequential palpation per rectum and plasma progesterone profiles indicate that ovarian cysts are dynamic and subject to structural and hormonal changes from time to time (Mujuni *et al.*, 1993).

Previous studies on experimentally induced ovarian cysts in cattle have depended upon palpation per rectum, ovariectomy or laparoscopy for monitoring the development and regression of cysts. Ultrasound monitoring is far more reliable and non-invasive for assessing ovarian cysts (Ribadu *et al.*, 1993), hence the objectives of the present study were to :-

(1) experimentally induce ovarian cysts in heifers using progesterone/oestradiol or ACTH treatment and to monitor cyst formation and development by ultrasonography and endocrine profiles.

(2) evaluate the LH response to exogenous oestradiol in heifers with experimentally-induced cysts because cows with spontaneously occurring cysts had variable responses (Dobson and Nanda, 1992).

MATERIAL AND METHODS

Nulliparous Holstein/Friesian heifers 12-18 months of age and weighing 313-411 kg were used. The heifers were observed in all the studies around the time of expected oestrus 3 times daily for mounting behaviour among the group. Indwelling jugular catheters were inserted a day before frequent sampling ($\leq$ 4 hourly) in all heifers.

Control

Oestrus was synchronised in 6 heifers using two injections of cloprostenol (2 ml Estrumate, Coopers, Pitman-Moore, Crewe, U.K.) 11 days apart. Ovarian ultrasonography and blood sampling (via tail venepuncture) was carried out daily through a complete interovulatory interval. Between day 19-22 of the oestrous cycle, the heifers were blood sampled every 4 h for LH measurement.

On another occasion, to characterise a control response to exogenous oestradiol, 1 mg oestradiol benzoate (Intervet, Cambridge, U.K.) in 2 ml arachis oil was given intramuscularly 24 h after a second cloprostenol injection in 8 heifers. Blood samples for LH were collected at the time of oestradiol injection (0 h) and every 2 h from 16-32 h after injection.

Ultrasound examination was carried out using an ultrasound scanner (SSD 210 DXII, Aloka, BCF Technology, Livingstone, U.K.) equipped with a 7.5 MHz rectal transducer. All follicles 5mm or more in diameter and corpora lutea (CL) were measured after freezing the image on the screen using in-built calipers.

Representative prints were made using an on-line thermal printer (Sony, Video Graphic UP -850, Japan).

A dominant follicle was defined as the largest follicle in the ovary 10 mm or more in diameter in the absence of other larger follicles (Savio *et al.*, 1990). A dominant follicle and its cohorts were defined as a wave. The appearance of follicles was defined as the first day a follicle attained $\geq$ 5 mm diameter. Cysts, as detected by ultrasonography, were defined as follicular structure(s) of 20 mm or greater in diameter present for 10 days without ovulation and CL development (Cook *et al.*, 1990, Chavette *et al.*, 1993). The first day of cyst formation was the day a follicle attained 20 mm or more in diameter.

An increase of LH concentration above 10 ng/ml in consecutive samples ($\leq$ 4 hourly) was considered an LH surge. The time of the first increase was considered as the onset of the surge.

Progesterone/oestradiol treatment

Eight heifers received subcutaneous injections of 15 mg oestradiol (Sigma Chemical Co. St Louis, U.S.A.) and 37.5 mg progesterone (Sigma Chemical Co., St Louis, U.S.A.) dissolved in 2 ml double distilled ethanol every 12 h for 7 days beginning on day 15 of the oestrous cycle (Cook *et al.*, 1990). Daily ovarian ultrasonography and blood sampling was carried out until ovulation or corpus luteum (CL) development or formation and regression of a cyst.

Ten days after cyst formation, heifers were challenged with 1 mg oestradiol benzoate in 2 ml arachis oil at time 0 h and sampled every 2 h from 16-32 h. In heifers with luteal cysts, cloprostenol was given 24 h before oestradiol to induce

luteolysis. The oestradiol challenge was repeated after another 10 days in heifers in which cysts were still present or new cysts had developed.

ACTH treatment

Seven heifers were synchronised for oestrus with cloprostenol as described earlier. Beginning on day 15 of the subsequent oestrous cycle, the heifers received subcutaneous injections of 100 iu ACTH (Tetracosactrin acetate (β^{1-24} Corticotrophin); Synacthen depot, CIBA, Horsham, England) every 12 h for 7 days. Ovarian ultrasonography and blood sampling was carried out daily from day 14 of the cycle until ovulation and CL development or formation and regression of ovarian cysts. In addition, around the time of expected oestrus (day 19-22) the heifers were bled every 4 h for LH measurement.

In heifers in which ovarian cysts developed, 1mg oestradiol benzoate in 2 ml arachis oil was given 10 days after cyst formation. Blood samples were obtained every 2 h from 16-32 h through indwelling jugular catheters.

Blood samples were centrifuged at 1000 g and stored at -20^{0}C until analysis for progesterone (Kanchev *et al.*, 1976), cortisol (Alam and Dobson, 1986) and luteinizing hormone (Alam and Dobson, 1986). The characteristics of these assays are given in chapter 2.

Statistics

The mean ($\pm$ S.E.M.) interovulatory interval, day of first appearance and diameter of ovulatory/cystic follicles were calculated for control, progesterone/oestradiol-treated and ACTH-treated heifers. The rates of growth of dominant ovulatory follicles and cystic follicles were calculated by regression

analysis. The linear regression lines (slopes in mm/day) were analysed for parallelism by Student's *t* test to determine if there were differences in growth rates. Mean interovulatory interval, maximal ovulatory follicle size and cortisol concentration were also compared in treated and control animals using Student's t-test.

RESULTS

Control

Typical examples of growth and regression patterns and progesterone profile in a heifer with 2 waves/cycle and a heifer with 3 waves/cycle during the control cycle are given in Figs 7.1 - 7.2 while Fig 7.3 represents the mean ($\pm$ S.E.M.) diameter of dominant nonovulatory, dominant ovulatory follicles and progesterone concentration in 4 heifers with 2 waves/cycle. Mean cycle length, day of appearance ($\geq$5 mm) of ovulatory follicles and maximal size of ovulatory follicles are presented in Table 7.1

A wave pattern of follicular growth was observed in all 6 control heifers. Four heifers had 2 dominant follicles per cycle with the second dominant follicle ovulating, while 2 heifers had 3 dominant follicles per cycle with the third dominant follicle ovulating. Dominant ovulatory follicles in heifers with 2 waves/cycle appeared on day 11 of the cycle while the dominant ovulatory follicle in heifers with 3 waves /cycle appeared on day 16 of the cycle.

The mean cycle length was 20.2 $\pm$ 0.7 days. One control heifer (C602) had one CL on each ovary during the control cycle. Peak plasma progesterone in this heifer was 8.2 ng/ml, similar to the peak plasma progesterone in the other heifers

with one CL during the cycle (6.4 ± 0.7 ng/ml). The mean progesterone concentration was low (<0.9 ng/ml) during the first few days of the cycle (day 1-3). It gradually increased with the formation of CL and reached peak amount on day -7 and declined rapidly on day -2. The mean cortisol values during the control cycle ranged from 4.2 to 11.2 ng/ml.

A preovulatory surge of LH was observed in 5 of 6 control heifers from samples taken every 4 h between days 19 and 22 of the control cycle. Oestrus behaviour was noted in all 6 heifers. Despite initial oestrus synchronization, subsequent spontaneous oestrus occurred early in one heifer hence the failure to detect the preovulatory LH surge in this heifer. An LH surge (>10 ng/ml) in response to exogenous oestradiol (given 24 h after cloprostenol) was obtained in all 8 heifers in the 2-hourly samples obtained 16-32 h after oestradiol. The delay time to LH surge onset was 19.0 ± 0.76 h (mean ± S.E.M, range 18-24 h). The duration of the surge was 4.8 ± 0.53 (range 4-6 h) with peak values of 32.0 ± 3.9 ng/ml (range 20.6-41.4 ng/ml). Progesterone concentration at the time of oestradiol injection was <0.9 ng/ml in all heifers.

Progesterone/oestradiol treatment

Individual follicular growth patterns and progesterone profiles are given in Figs 7.4-7.11 while Fig 7.12 and 7.13 are representative ultrasonograms of a steroid-induced follicular and luteal cysts. Mean (± S.E.M.) interovulatory interval, day of appearance of ovulatory/cystic follicles, and length of static phases are presented in Table 7.1.

A static phase (period during which follicles 5mm or more or luteal tissue were absent in both ovaries) ranging between 3 and 16 days was observed in all 8

heifers. Normal oestrus behaviour was not exhibited at the time of expected oestrus. After the static phase, a period of follicular growth (follicular phase) occurred in all cows which resulted in ovulation in 5 and development of cysts in 3. The dominant follicle of the first wave ovulated in 3 of 5 heifers while the dominant follicle of the second wave ovulated in 2 of 5 heifers.

Of 3 heifers that developed ovarian cysts, one was follicular with a thin wall < 3 mm and two were luteal with thick walls > 3 mm. In the heifer with a follicular cyst, a second cyst developed on the same ovary as the first one regressed. Intermittent oestrus behaviour occurred in the heifer with the follicular cyst whereas the two heifers with luteal cysts were anoestrus.

Progesterone concentration decreased to <0.9 ng/ml in most heifers 3 days after the last steroid injection and remained low (<0.9 ng/ml) for an average of 15-25 days in the noncystic group but increased following ovulation and CL formation. In the heifer with a follicular cyst, progesterone was low for 62 days. The heifers with luteal cysts had progesterone concentration of 1.1 to 8.2 ng/ml.

The interovulatory interval was significantly extended ($P<0.05$) in both the noncystic and cystic group compared to the control heifers. The day that ovulatory follicles appeared in noncystic heifers and cystic follicles were significantly ($P<0.05$) delayed compared to the ovulatory follicles in control cycle. There was no difference ($P>0.05$) in maximal size of the follicle which eventually ovulated in the noncystic heifers compared to the controls. The cyst size was significantly ($P<0.05$) greater than ovulatory follicles. The mean growth rates of the noncystic ovulatory follicle and cystic follicles were not different ($P>0.05$) from growth rates of control ovulatory follicles.

Short luteal phases (with interovulatory intervals of 7-8 days) were observed in 2 heifers in the noncystic group. Maximum progesterone concentration was also subnormal (1-2 ng/ml).

A surge of LH was elicited following an oestradiol challenge at the time of the second follicular cyst in heifer C602. The time delay after oestradiol to the onset of the surge was 24 h. Peak LH concentration was 23.6 ng/ml. Exogenous oestradiol failed to elicit an LH surge during the existence of the first follicular cyst in C602 and in the 2 heifers with luteal cysts even though cloprostenol had been administered 24 h earlier.

ACTH treatment

Mean ($\pm$ S.E.M.) interovulatory interval, day of appearance of ovulatory/cystic follicle, maximal size of ovulatory/cystic follicle, growth rates of ovulatory/cystic follicles are summarised in Table 7.1. Individual follicular growth patterns and progesterone profile are given in Figs 7.14 - 7.20 while Fig 7.21 is a composite representation of dominant ovulatory/cystic follicle and endocrine profiles in the control and ACTH-treated heifers.

No static phase was observed after ACTH treatment. The interovulatory interval was extended in all 7 heifers. Three heifers developed ovarian follicular cysts at about day 25 (day 0 = day of oestrus). The follicles that became cystic in these 3 heifers were all present at the initiation of ACTH treatment. The growth rate of ovulatory follicles in noncystic heifers and cystic follicles were not significantly different ($P>0.05$) from ovulatory follicles in control cycle. The cyst diameter persisted at 20-25 mm for 10 days or more before regression started. Four heifers failed to develop ovarian cysts and ovulatory follicles appeared on day 19.0 $\pm$ 1.1

culminating in ovulation between days 28 and 31. The maximum size of these ovulatory follicles were significantly larger ($P<0.05$) than control ovulatory follicles.

Cortisol concentration increased significantly ($P<0.05$) within 24 h after starting ACTH treatment and remained high (> 20 ng/ml) for about 14 days. Progesterone concentration remained elevated for 7 more days in all ACTH-treated heifers compared to control cycles. After the decline in progesterone concentration by day 24, values remained low in the treated noncystic group until ovulation and CL formation. Plasma progesterone concentration in the cystic group was also low (<0.9 ng/ml) until cyst regression and CL development after subsequent ovulation (day 36-43).

Oestrous behaviour was absent at the time of expected oestrus in all ACTH-treated heifers. None of the 3 heifers that developed follicular cysts showed exaggerated sexual behaviour.

A short luteal phase lasting about 3 days with an interovulatory interval of 6 days was observed in 1 heifer after regression of a follicular cyst.

Only 2 (H263 and H283) of 3 heifers with follicular cysts responded by eliciting an LH surge after exogenous oestradiol given on day 10 after cyst formation. The time delay after oestradiol to the onset of the surge was 20 ± 2.0h. Peak concentration was 15.1 ± 0.9 ng/ml with a mean duration of 5.0 ± 1.0 h.

The mean diameters of ovulatory (control and steroid-treated non-cystic), dominant nonovulatory (control and steroid-treated noncystic) and dominant follicles that became cystic in treated heifers are given in Figs 7.22-7.24. The growth rate of ovulatory follicles (during the last 6 days) in both progesterone/oestradiol and

ACTH-treated (non-cystic) heifers were not different ($P>0.05$) from ovulatory follicles of control cycles. However, having attained 5 mm, the ovulatory follicles in controls and after ACTH took 10-11 days to ovulate whereas the ovulatory follicles in progesterone/oestradiol treated heifers took 7 days. Initially, there was slower growth of control ovulatory follicles - longer growing from 5 to 8 mm than both treated groups. Dominant nonovulatory follicle growth similar for control and progesterone/oestradiol treated heifers although existence of latter lasted 4 days longer. After both cyst induction treatments, cyst growth was similar (whether follicular or luteal) for first 20 days to reach 20-25 mm. ACTH-induced cysts then regressed quite quickly (in 4 days) whereas progesterone/oestradiol induced cysts retained diameter >20 mm for further 8 days, followed by 6 day regression phase.

DISCUSSION

During the control oestrous cycle, 2 waves of follicular growth were observed in 4 heifers and 3 waves of follicular growth in 2 heifers. A cycle with 2 waves is consistent with the results of Knopft *et al.* (1988) and Ginther *et al.* (1989) while 3 waves per cycle supports the findings of Ireland and Roche (1983); Fortune *et al.* (1988); Savio *et al.* (1988) and Ireland and Roche (1987). Cycles with 3 waves of follicular development seemed to have longer luteal phases than those with 2 waves, suggesting that the number of waves in a cycle is determined by the time of luteal regression or vice-versa. Each wave of follicular development was characterised by simultaneous emergence (from a pool of small follicles) of a cohort of growing follicles >5 mm in diameter. One of this group rapidly emerged as the dominant follicle and continued to grow while the others became atretic and

regressed. A maximum size of 15 mm was attained by dominant follicles in the control animals of the present study. The first dominant follicle in the heifers with 2 waves/cycle and the first and second dominant follicles in those with 3 waves/cycle became atretic and regressed after a few days; they were replaced by a further dominant ovulatory follicle from the next wave. This observation concurs with previous studies (Fortune *et al.*, 1988; Savio *et al.*, 1988; Webb *et al.*, 1992).

The growth rate of the dominant ovulatory follicle in control heifers (1.5mm/day) is similar to 1.4mm/day reported by Knopft *et al.* (1989).

Despite hormone synchronization, spontaneous oestrus occurred early in 1 heifer, hence the failure to detect a preovulatory surge of LH in this animal. Preovulatory surges of LH were detected in the remaining 5 heifers in at least 2 consecutive 4-hourly samples collected around the oestrus period (days 19-22).

The observation of a static phase in all 8 heifers treated with progesterone/oestradiol was similar to the result of Cook *et al.* (1990). The static phase observed in the present study (3-16 days) was, however, shorter than the 29-41 days reported previously (Cook et *al.*, 1990). The methods of monitoring of ovarian structures may account for the differences in the length of the static phases across these studies. In the previous study, follicular structures were detected by palpation per rectum. The formation of a new follicular cyst as another one regressed in heifer C602 after progesterone/oestradiol treatment confirms previous reports (Kesler *et al.*, 1980; Cook *et al.*, 1990) that ovarian cysts are dynamic structures.

It should be noted that, even though the heifers were synchronised and given the same steroid treatment, there was diversity in the types of cysts formed;

follicular in 1 heifer and luteal in 2 heifers. It is not clear why different ovarian pathological responses developed in heifers under the same treatment. Cook *et al.* (1990) observed 3 different histological types of cysts in cows subjected to the same steroid regimen. The steroidogenic ability of cysts depends on the development and viability of granulosa or luteinised thecal tissue (Choi *et al.*, 1982).

In the 7 ACTH-treated heifers, follicular cysts were induced in 3 out of 7 (43%) heifers. This is similar to the result of Refsal *et al.* (1987) who reported experimental induction in 2 out of 5 (40%) cows using a similar protocol. It contrasts however, with the result of Liptrap and MacNally (1976) who obtained cysts in 5 out of 5 cows.

Although luteal regression was noted in the ACTH-treated heifers during ultrasonography, this did not result directly in ovulation as would have been expected in a normal cycle; instead the cycle was extended with ovulation or development of ovarian cysts occurring much later (8-10 days).

Treatment of cows with Receptal (a synthetic GnRH agonist, Buserelin; Hoechst-Roussel, NJ) led to alteration in follicular distribution with increased frequency of cloudy follicles as observed by ultrasonography (Thatcher *et al.*, 1989). Cloudy follicles were not observed during ultrasonography in both progesterone/oestradiol and ACTH-treated heifers in the present study.

The continuous elevation of plasma progesterone concentration after ACTH treatment may have been due to progesterone derived from the adrenal gland (Watson and Munro, 1984). The prolonged progesterone secretion from the adrenal gland may decrease the pulse release rate of GnRH from the hypothalamus and, or, prevent the positive feedback effects of oestradiol on the pituitary (Hansel and

Convey, 1983; Peters, 1986; Moberg, 1987). It may also contribute to delaying the onset of oestrus or of cyst formation by suppression of the synthesis and release of uterine $PGF_2\alpha$ (Porter and Behrman, 1972; Challis *et al.*, 1976) and, or, inhibition of endometrial oxytocin receptor formation (McCracken *et al.*, 1984).

From the mean diameter of ovulatory follicles, dominant non-ovulatory (in both controls and treated non-cystic heifers) and cystic follicles in treated (cystic) heifers (Fig 7.20-7.22), it can be seen that the "decision" as to whether a dominant follicle was going to be non-ovulatory was made early (4 days) after its emergence. However, a "decision" as to whether ovulation or cyst formation would ensue was delayed (7-11 days) after emergence ($\geq$ 5 mm). It is not known what factors influence the "decision" as to whether ovulation or persistence of the dominant follicle as a cyst should occur.

Although a preovulatory LH surge was not observed in all 7 ACTH-treated heifers around the expected oestrus period, only 3 developed ovarian cysts. Cortisol, ACTH or progesterone may have altered the LH pulse frequency at such a time that ovulation was achieved in 4 heifers whereas there was persistence of the dominant follicle into a cystic structure in the remaining 3 heifers. The precise requirement for LH pulses frequency by dominant follicles in cattle is yet unknown. Haresign *et al.* (1983) has indicated that an increase in mean circulating concentration of LH rather than the episodic pattern may be important at the ovarian level. In addition, it is worth noting that each of the follicles that developed into ovarian cysts were >5 mm at the beginning of ACTH treatment.

Overall, 3 out of 6 heifers with cysts (in both treatment groups) responded to exogenous oestradiol with an LH surge. It is generally agreed that the feed-back

regulation of oestradiol on hypothalamic-pituitary axis is altered in cystic ovarian degeneration (Zaied *et al.*, 1981; De Silva and Reeves, 1988). The nature of the alteration is poorly understood. It is probable that oestradiol concentration (though not determined in this study) was high in heifers that failed to respond to exogenous oestradiol. Savio *et al.* (1990) noted higher oestradiol concentration during the early (functional) phase than in the later (structural) phase of cyst formation. Refsal *et al.* (1987) reported an LH surge in only 1 out of 4 cows with experimentally induced cysts. Approximately 50% of cows with spontaneous cysts challenged with oestradiol had a subsequent LH surge (Nanda *et al.*, 1991; Dobson and Nanda, 1992). It is still not clear why cows with cysts probably secreting oestradiol, do not respond to either endogenous or exogenous oestradiol. Presumably, once endogenous oestradiol concentrations decrease and conditions change (in as yet unknown manner), they can then respond to exogenous oestradiol by producing a preovulatory LH surge.

Short luteal phases were observed in 2 heifers in the progesterone/oestradiol treated group and 1 heifer in the ACTH-treated group. These treatments may provide models for detailed studies into the phenomenon of short luteal phases in cattle. It has been documented that short luteal phases occur in cattle and sheep during puberty, at spontaneous and gonadotrophin-induced ovulations postpartum and at the start of the breeding season in ewes (Lauderdale, 1986; Tegegne *et al.*, 1993).

The mechanism associated with subnormal luteal function (i.e. early luteolysis) in 3 heifers (2 progesterone/oestradiol treated and 1 in ACTH-treated) in the present study may be related to inadequate preovulatory follicular

development. This may result in inadequate stimulation of follicular cells by gonadotrophins which might hinder the ability of those cells to subsequently secrete progesterone (Garverick and Smith, 1986). Premature release of prostaglandin $F_2\alpha$ from the uterus may also be involved (Hunter, 1991).

In conclusion, the development of experimentally induced ovarian cysts was monitored by ultrasonography and endocrine profile. Different types of cysts may be induced with the same treatment. Ovarian cysts are dynamic structures. Response of induced cysts to exogenous oestradiol was variable.

Table 7.1: Characteristics of dominant ovulatory follicle/cyst development in heifers during a control cycle (n=6), progesterone/oestradiol (n=8) or ACTH treatment (n=7)

Parameter	Control (n=6)	Progesterone & oestradiol treatment		ACTH treatment	
		NC (n=5)	C (n=3)	NC (n=4)	C (n=3)
Inter-ovul. (days)	$^{a}20.2 \pm 0.7$	$^{b}44.2 \pm 2.6$	$^{c}83.0 \pm 2.1$	$^{d}29.8 \pm 0.9$	$^{e}36.7 \pm 2.2$
Ovul./cystic F appear. (day)	$^{a}12.2 \pm 1.3$	$^{b}36.0 \pm 2.3$	$^{b}36.0 \pm 1.5$	$^{c}19.0 \pm 1.1$	$^{a}14.0 \pm 0$
Maximal size (mm)	$^{a}14.0 \pm 0.7$	$^{a}14.4 \pm 1.2$	$^{b}28.7 \pm 1.9$	$^{c}19.5 \pm 1.6$	$^{c}23.7 \pm 0.7$
Growth rate (mm/day)	1.5 ± 0.1	1.5 ± 0.1	1.5 ± 0.2	1.8 ± 0.2	1.5 ± 0.2
Static phase (days)	-	12.0 ± 1.6	12.0 ± 4.5	-	-

NC = noncystic C = cystic

Within a row values with different superscripts are significantly different ($P<0.05$)

Fig 7.1: Patterns of growth and regresion of functional ovarian structures and progesterone (P4) profile during control oestrous cycle (H286)

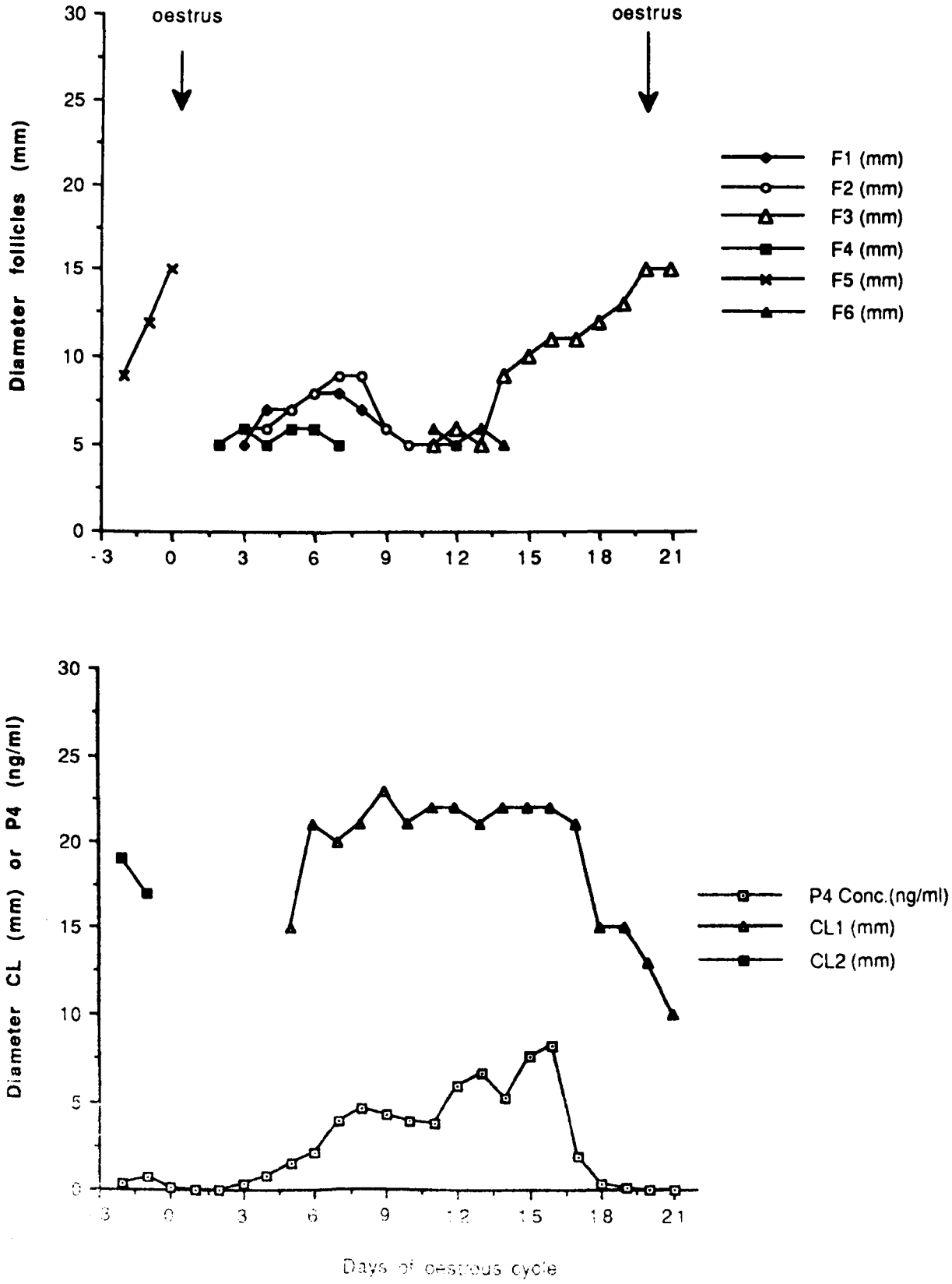


Fig 7.2: Patterns of growth and regression of functional ovarian structures and progesterone (P4) profile during control oestrous cycle (H2283)

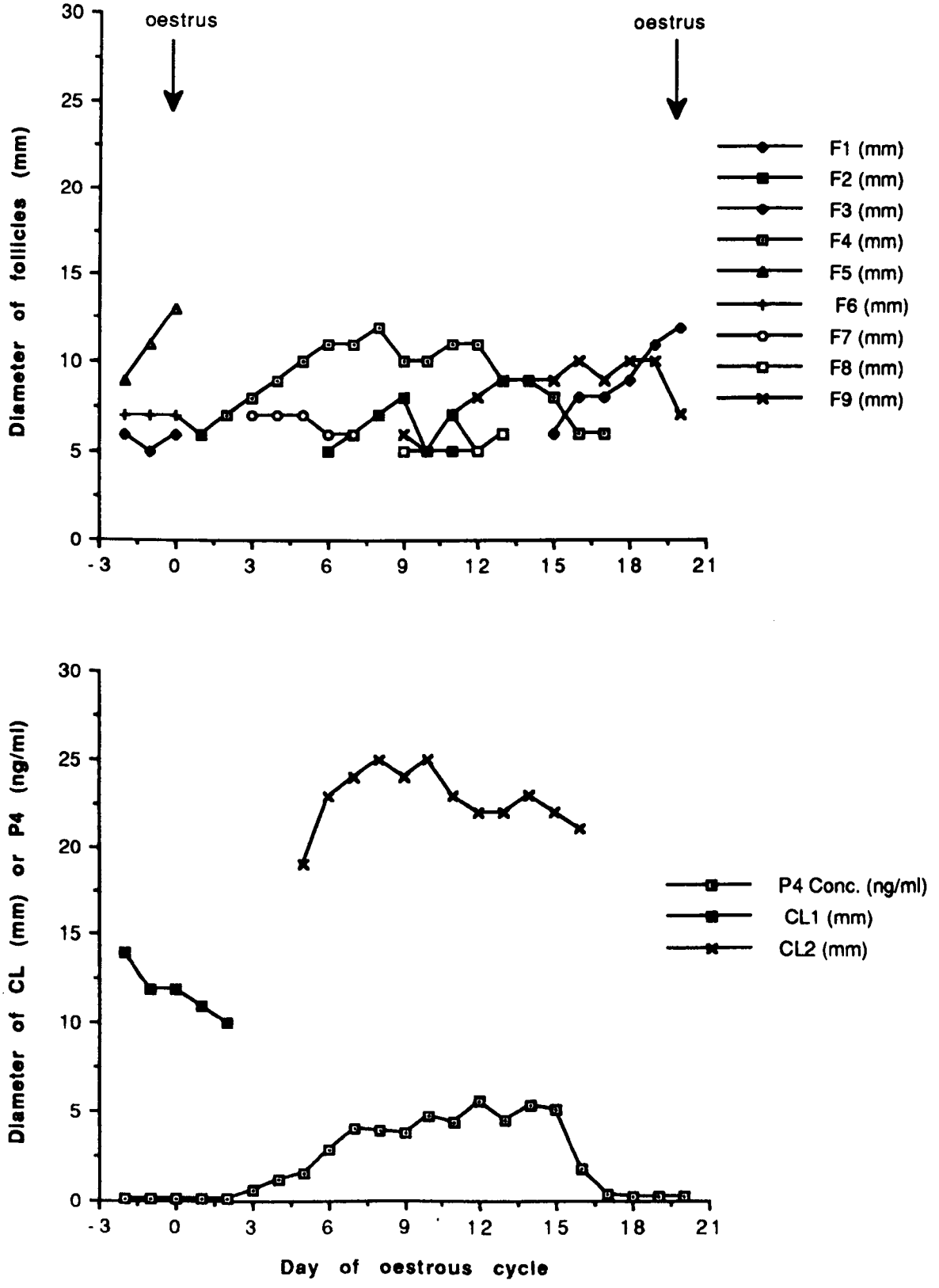


Fig 7.3: Mean (±S.E.M.) diameter of dominant non-ovulatory, dom. ovulatory follicle, CL and progesterone (P4) concentration during control oestrous cycles in 4 heifers with 2-waves/cycle

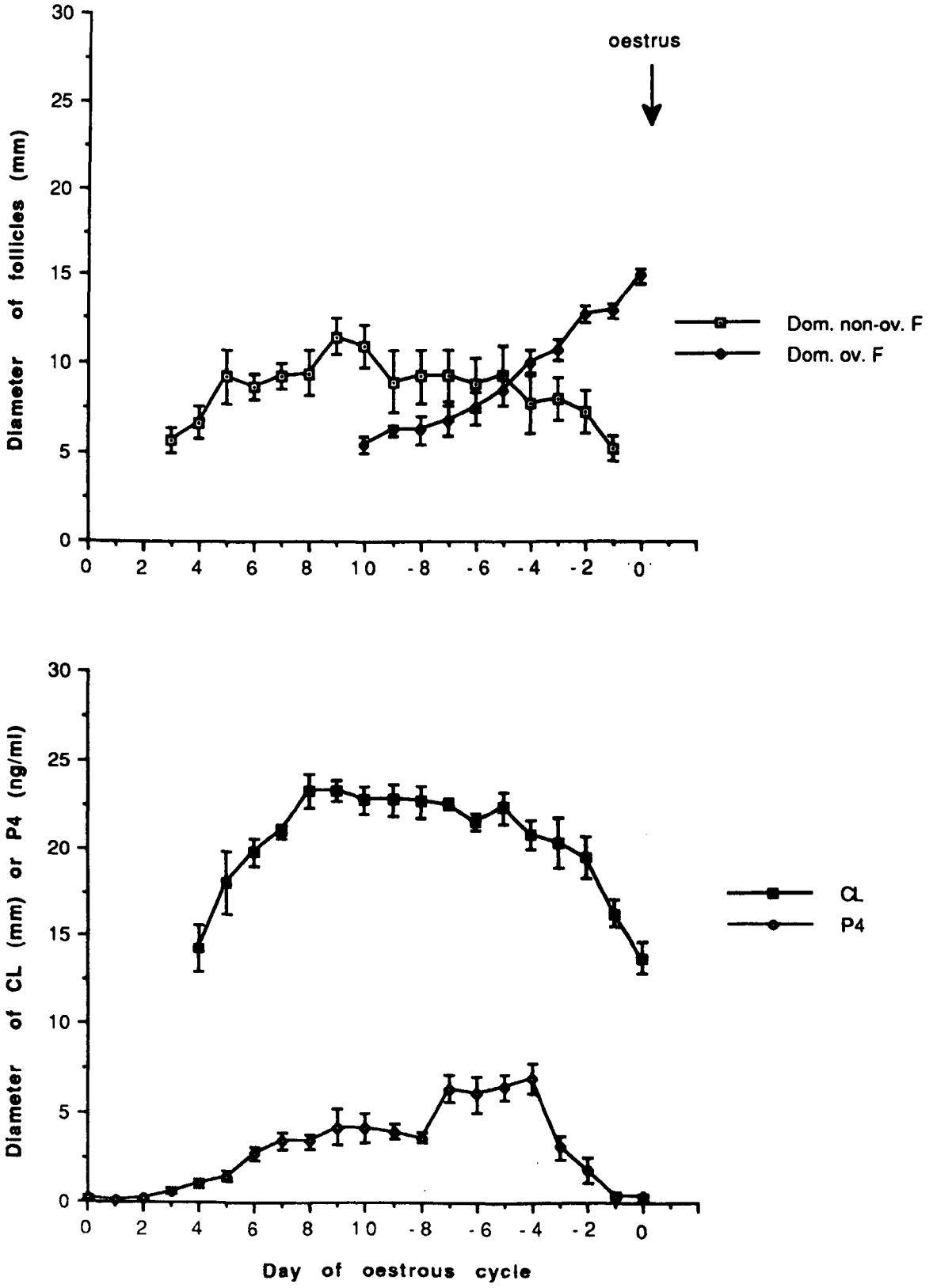


Fig 7.4: Changes in plasma progesterone (P4) and functional ovarian structures following treatment with progesterone/oestradiol. Horizontal bar represents period of P4/OE2 treatment and the horizontal line represents length of the static phase (H263).

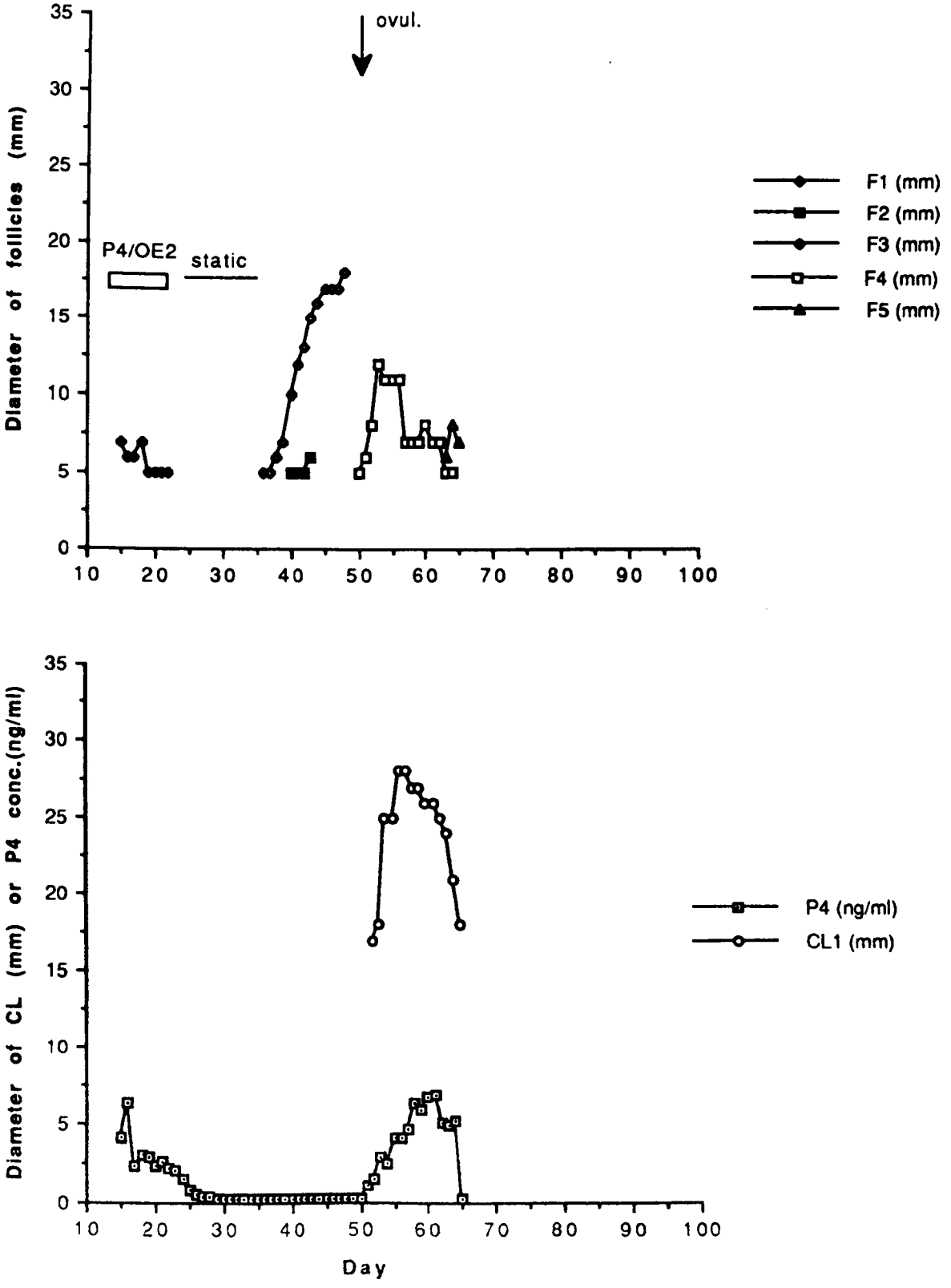


Fig 7.5: Changes in progesterone (P4) and functional ovarian structures following treatment with progesterone/oestradiol. Horizontal bar represents period of P4/OE2 treatment and the horizontal line represents length of the static phase (H276).

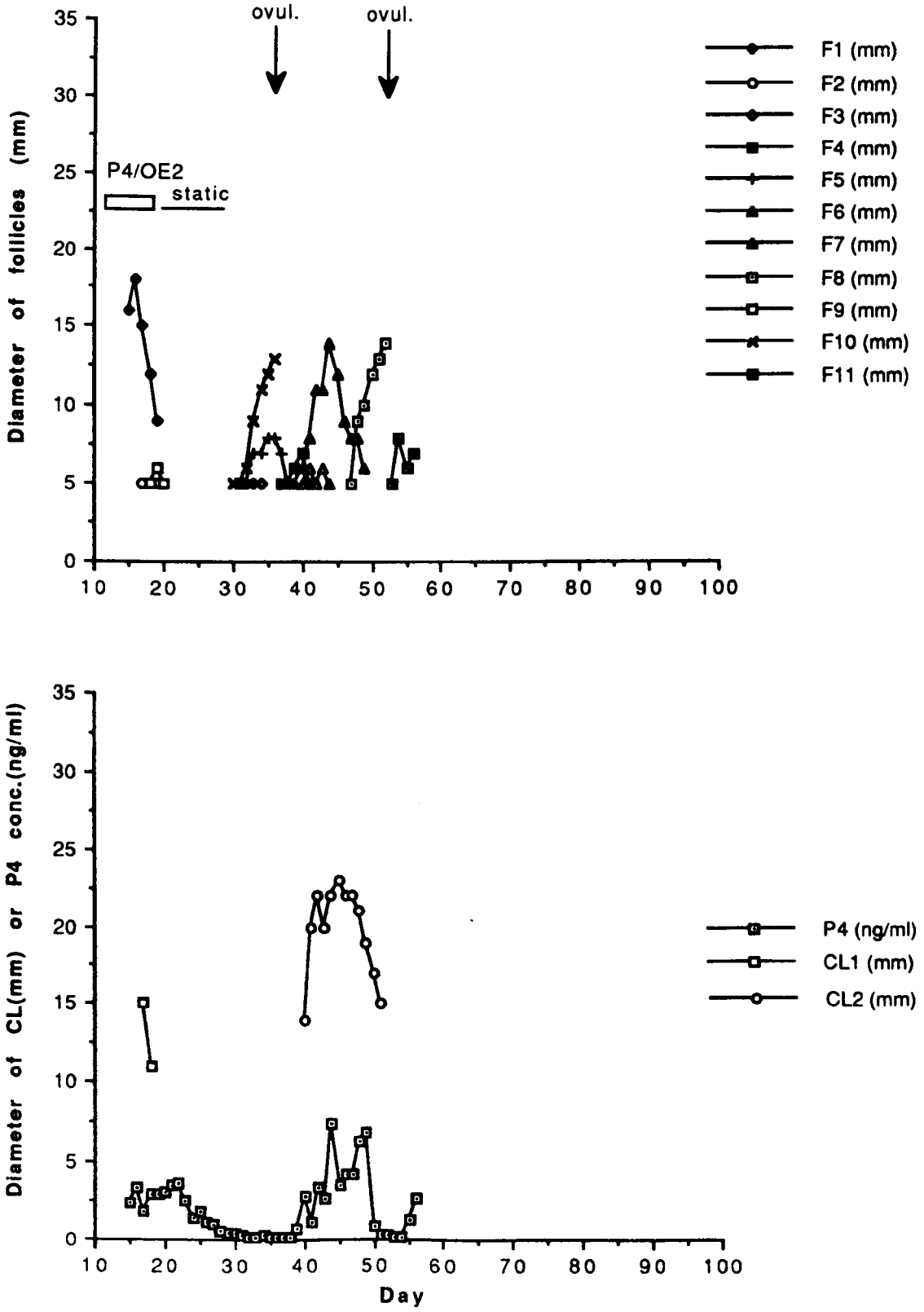


Fig 7.6: Changes in progesterone (P4) and functional ovarian structures following treatment with progesterone/oestradiol. Horizontal bar represents period of P4/OE2 tratment and the horizontal line represents length of the static phase (H283).

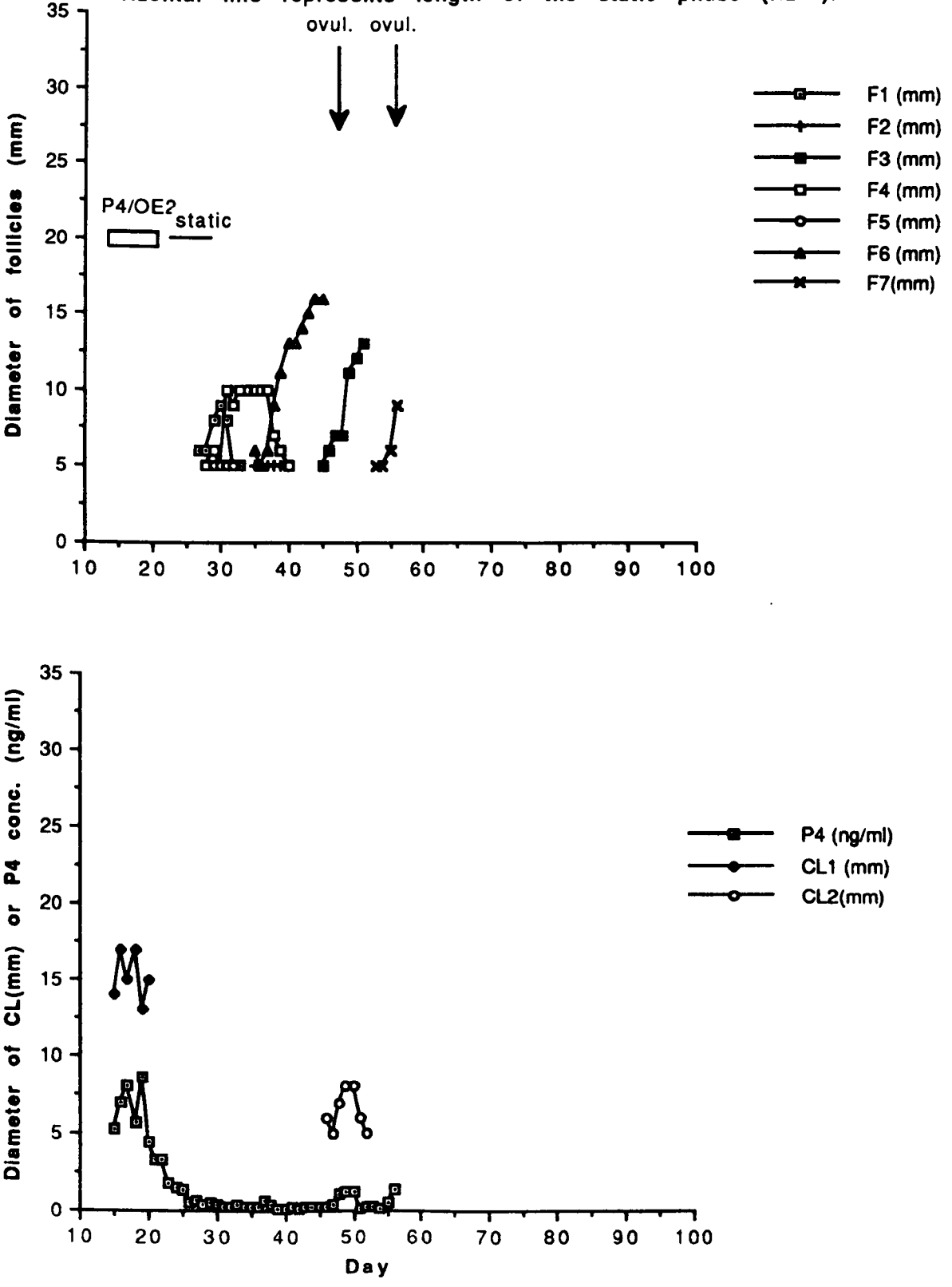


Fig 7.7: Changes in progesterone (P4) and functional ovarian structures following treatment with progesterone/oestradiol. Horizontal bar represents period of P4/OE2 treatment and the horizontal line represents length of static phase (H286).

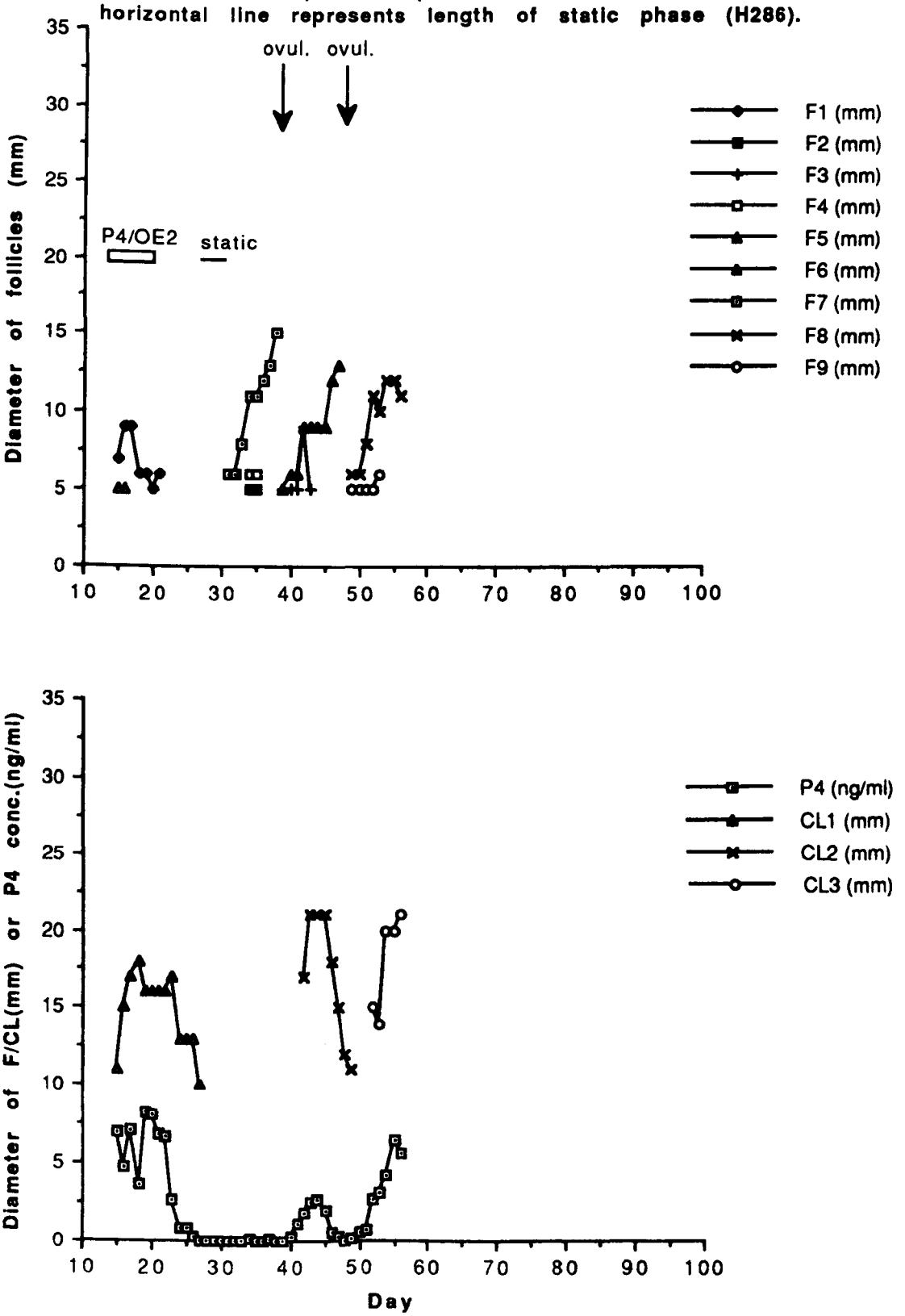


Fig 7.8: Changes in progesterone (P4) and functional ovarian structures following treatment with progesterone/oestradiol. Horizontal bar represents period of P4/OE2 treatment and the horizontal line represents length of the static phase (H2283).

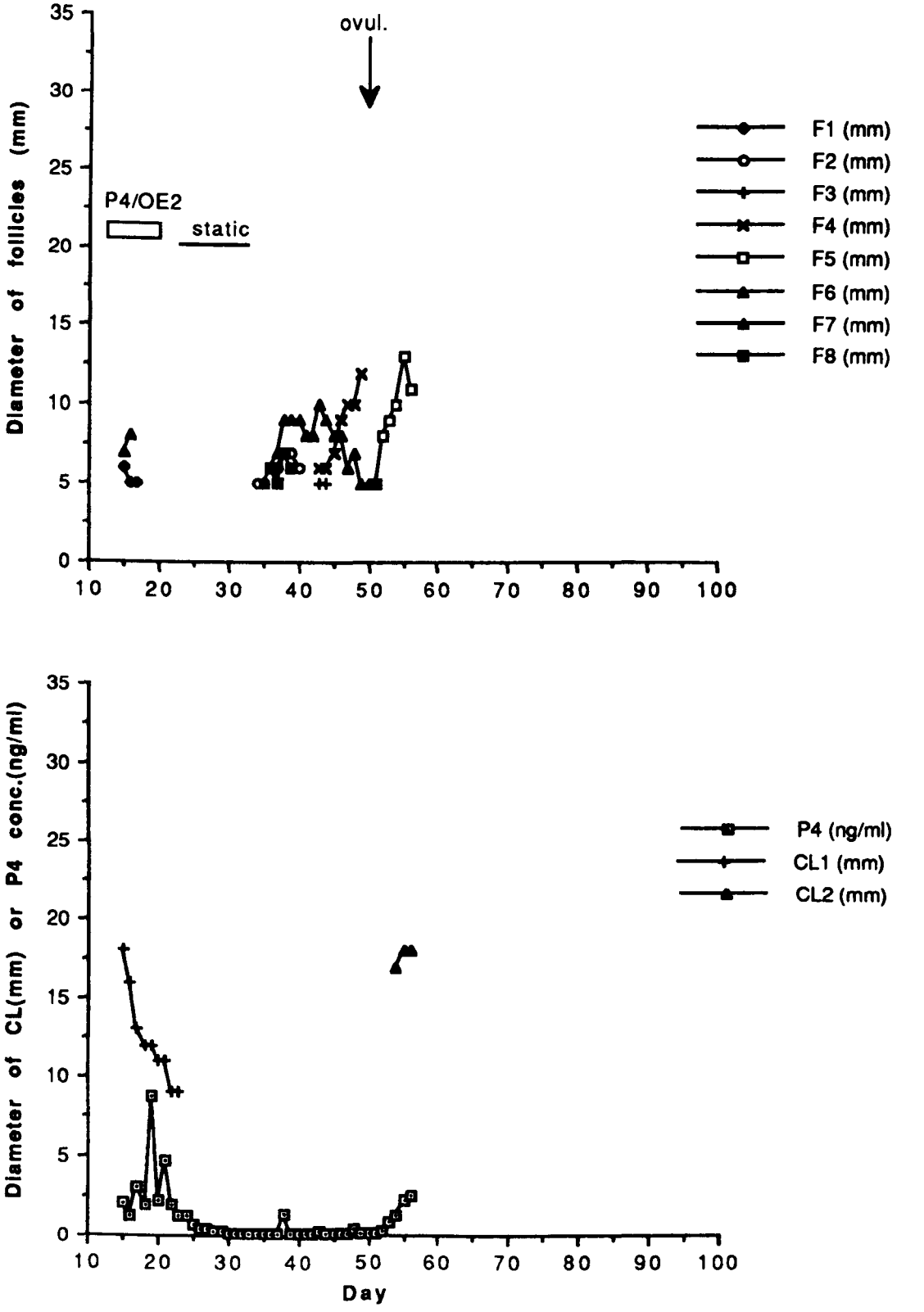


Fig 7.9: Patterns of growth and regression of follicular cysts (FC) and other ovarian structures following treatment with progesterone/oestradiol. Horizontal line represents length of static phase or cyst existence where indicated (C602).

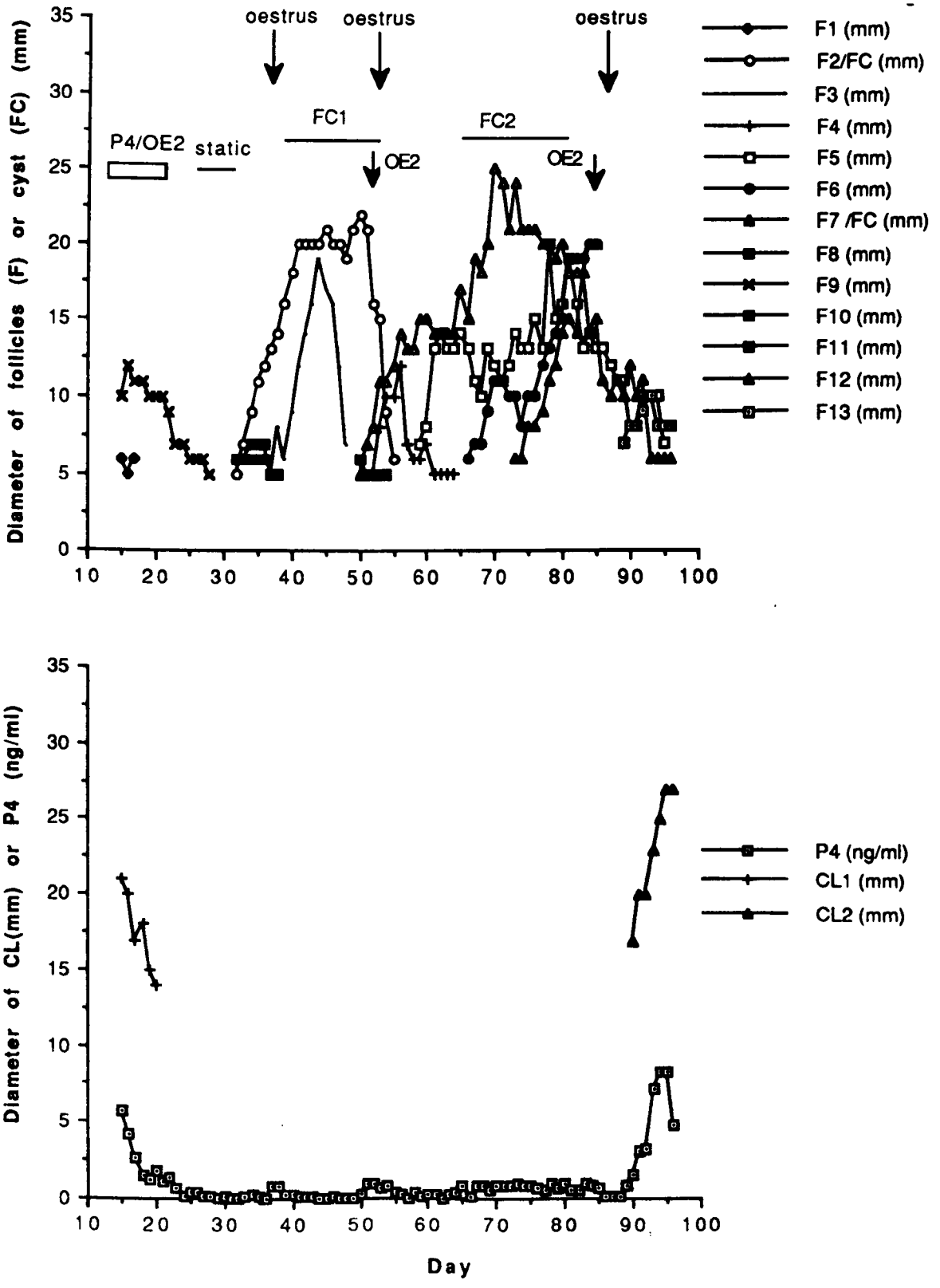


Fig 7.10: Changes in progesterone (P4) and ovarian structures following treatment with progesterone/oestradiol. Horizontal bar represents period of P4/OE2 treatment and the horizontal line represents length of static phase or cyst existence (H636).

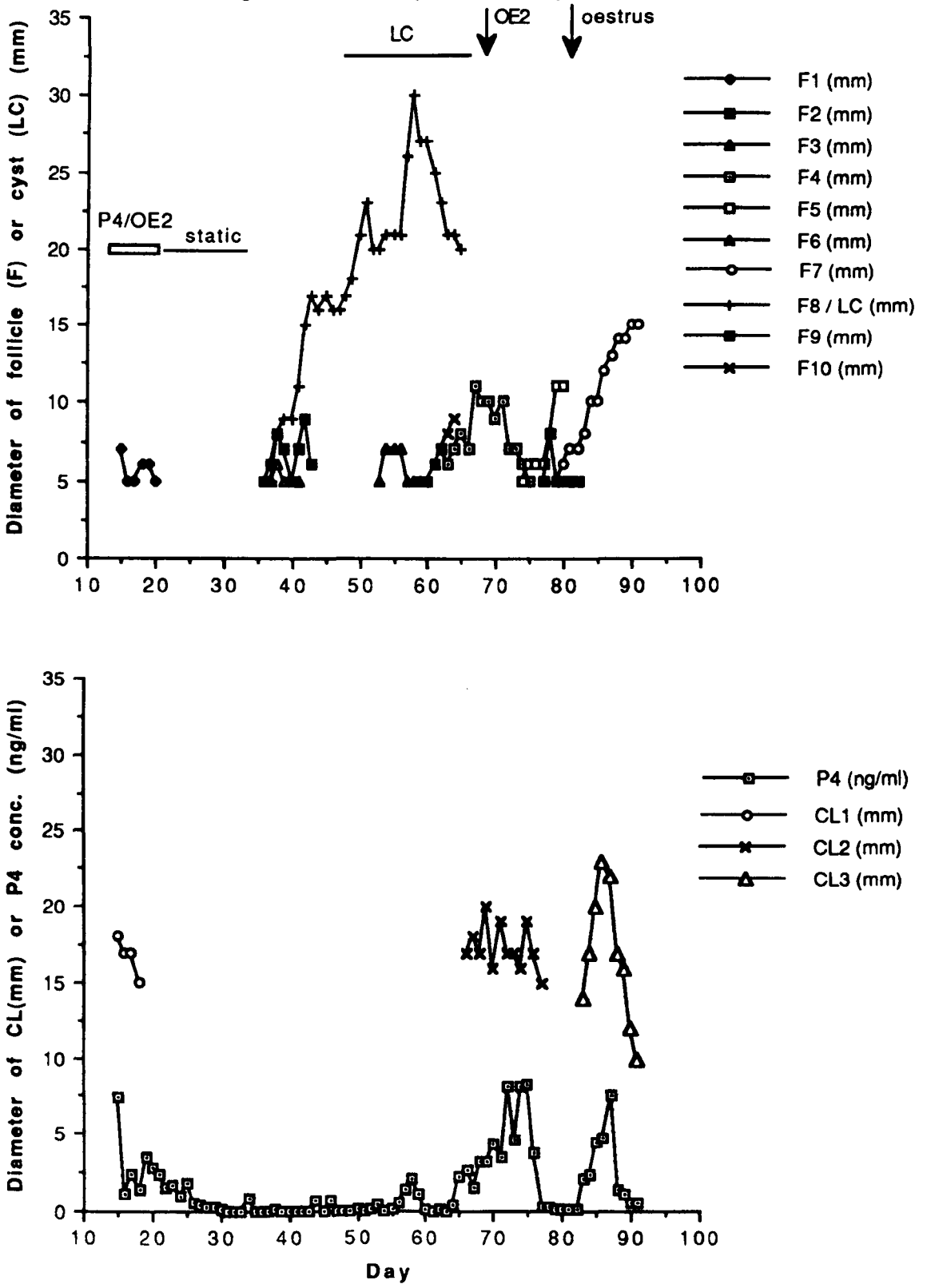
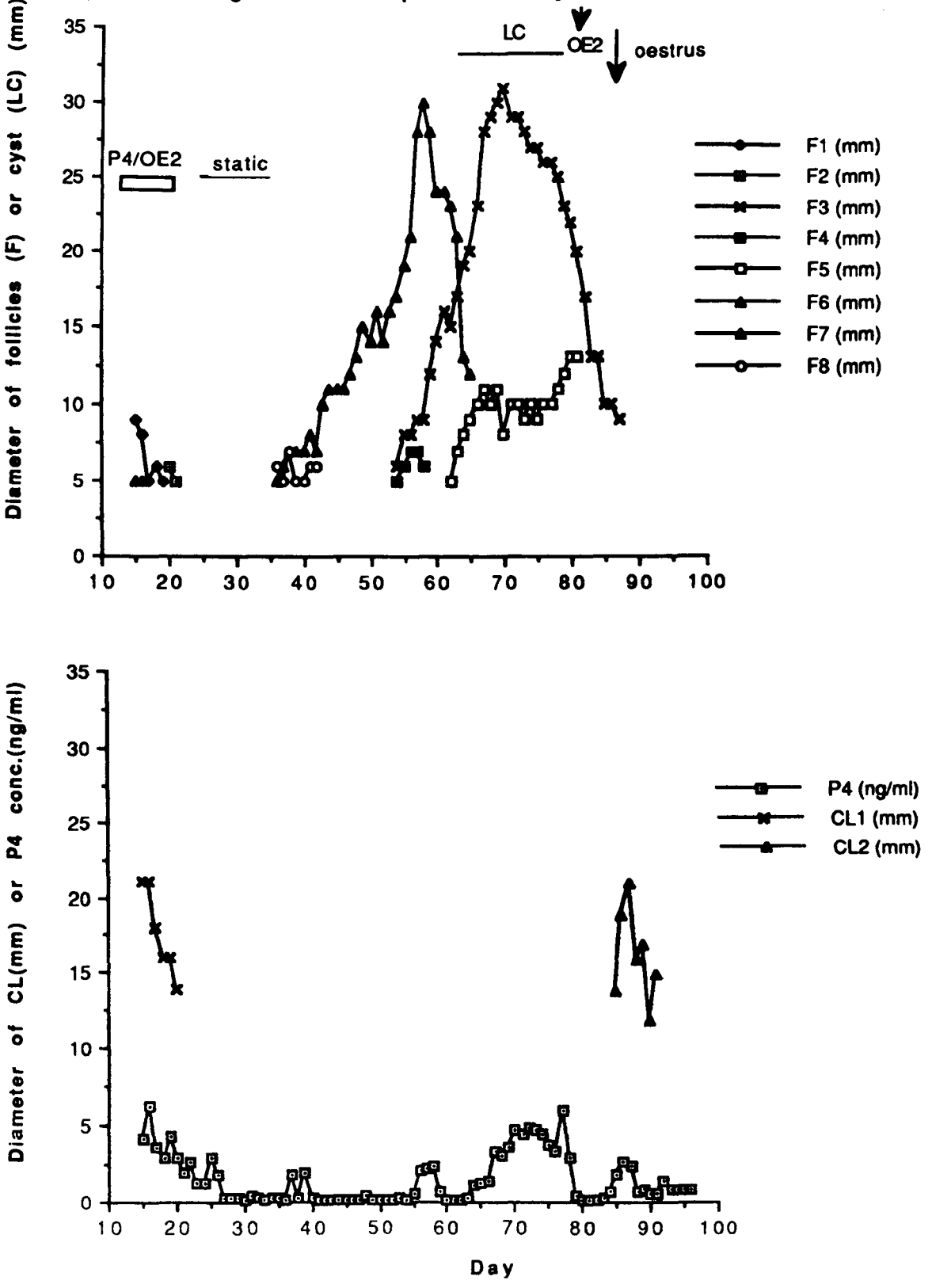


Fig 7.11: Changes in progesterone (P4) and ovarian structures following treatment with progesterone/oestradiol. Horizontal bar represents period of P4/OE2 treatment and the horizontal line represents length of static phase and cyst existence (H638).



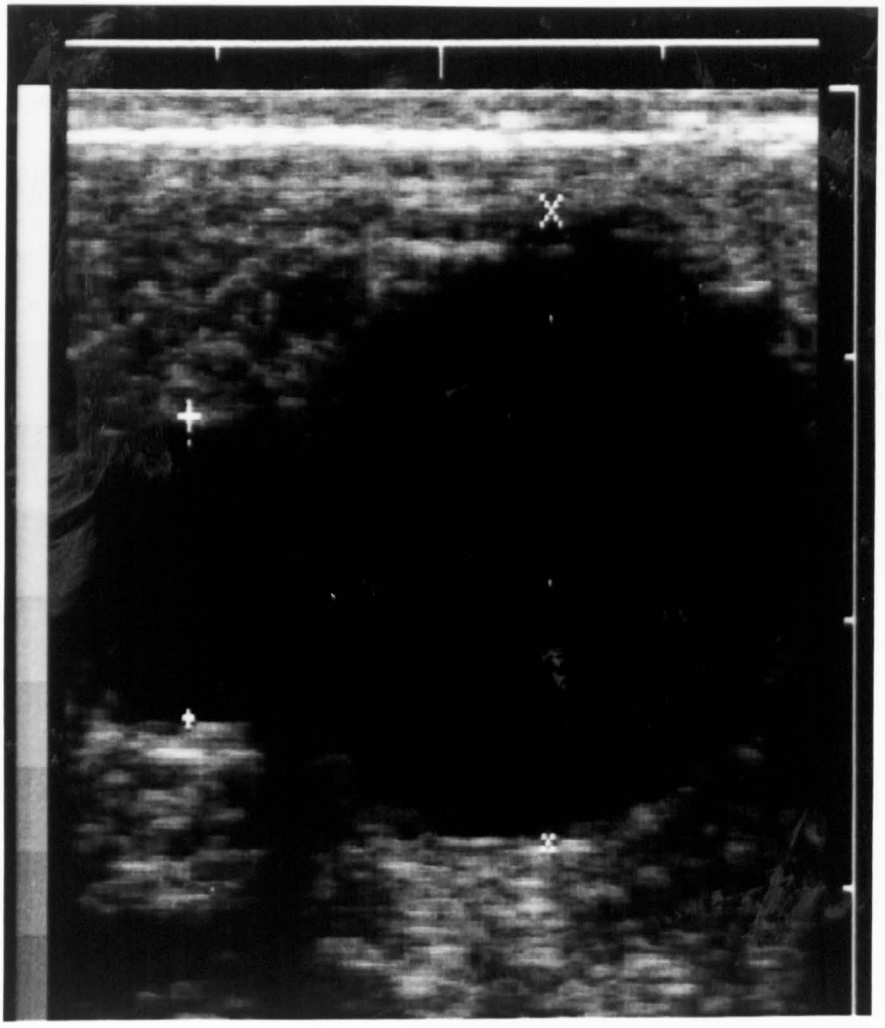


Fig 7.12: Ultrasonogram of a steroid-induced follicular cyst (P_4/E_2)

Cyst cavity diameter = 24 mm

Cyst diameter = 26 mm

diameter of follicle (+...+) = 11 mm

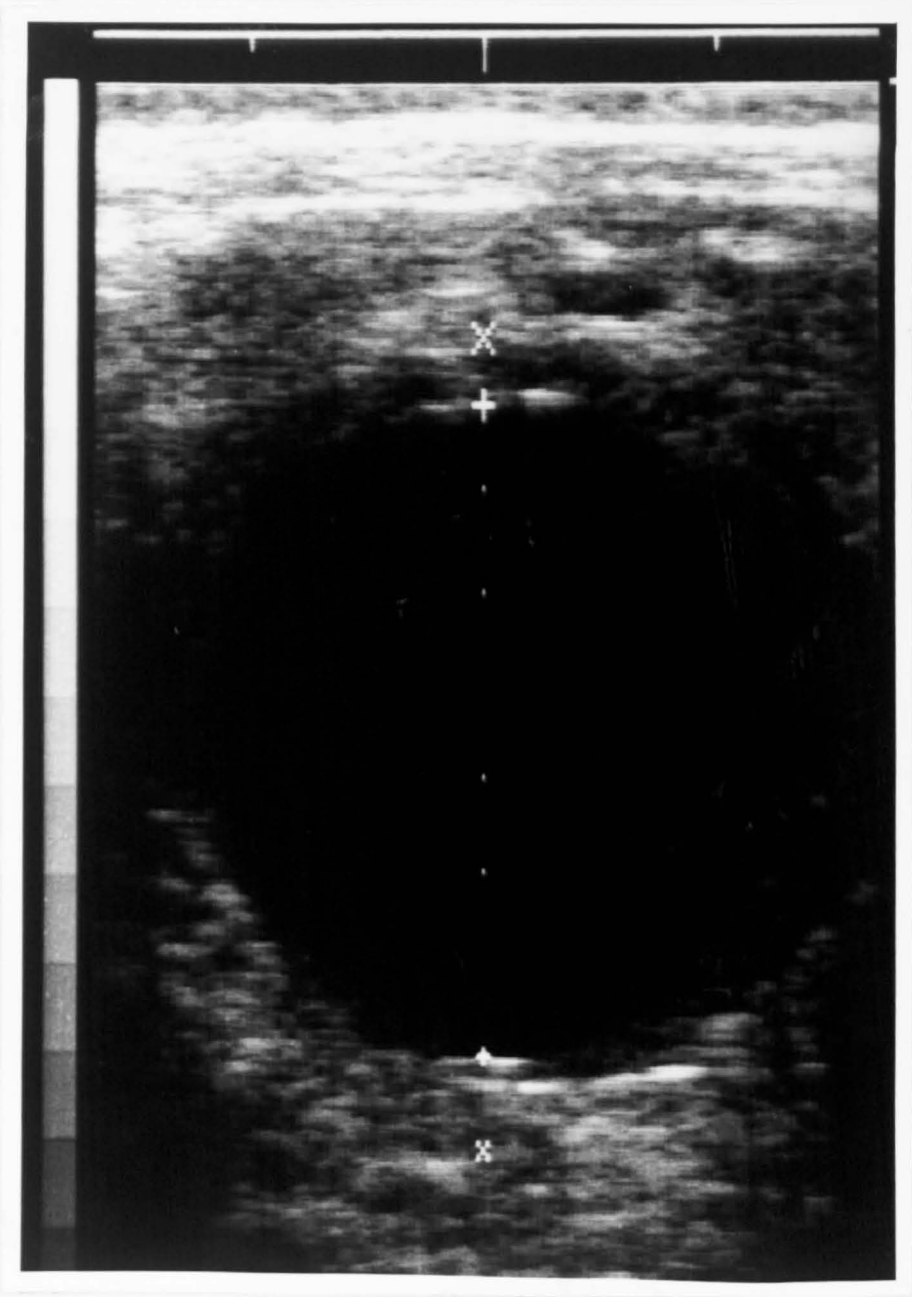


Fig 7.13: Ultrasonogram of a steroid-induced luteal cyst (P_4/OE_2)

Cyst cavity diameter = 23 mm

Cyst diameter = 30 mm

Fig 7.14: Patterns of growth and regression of ovarian structures and progesterone (P4) profile following treatment with ACTH as depicted by horizontal bar (H276)

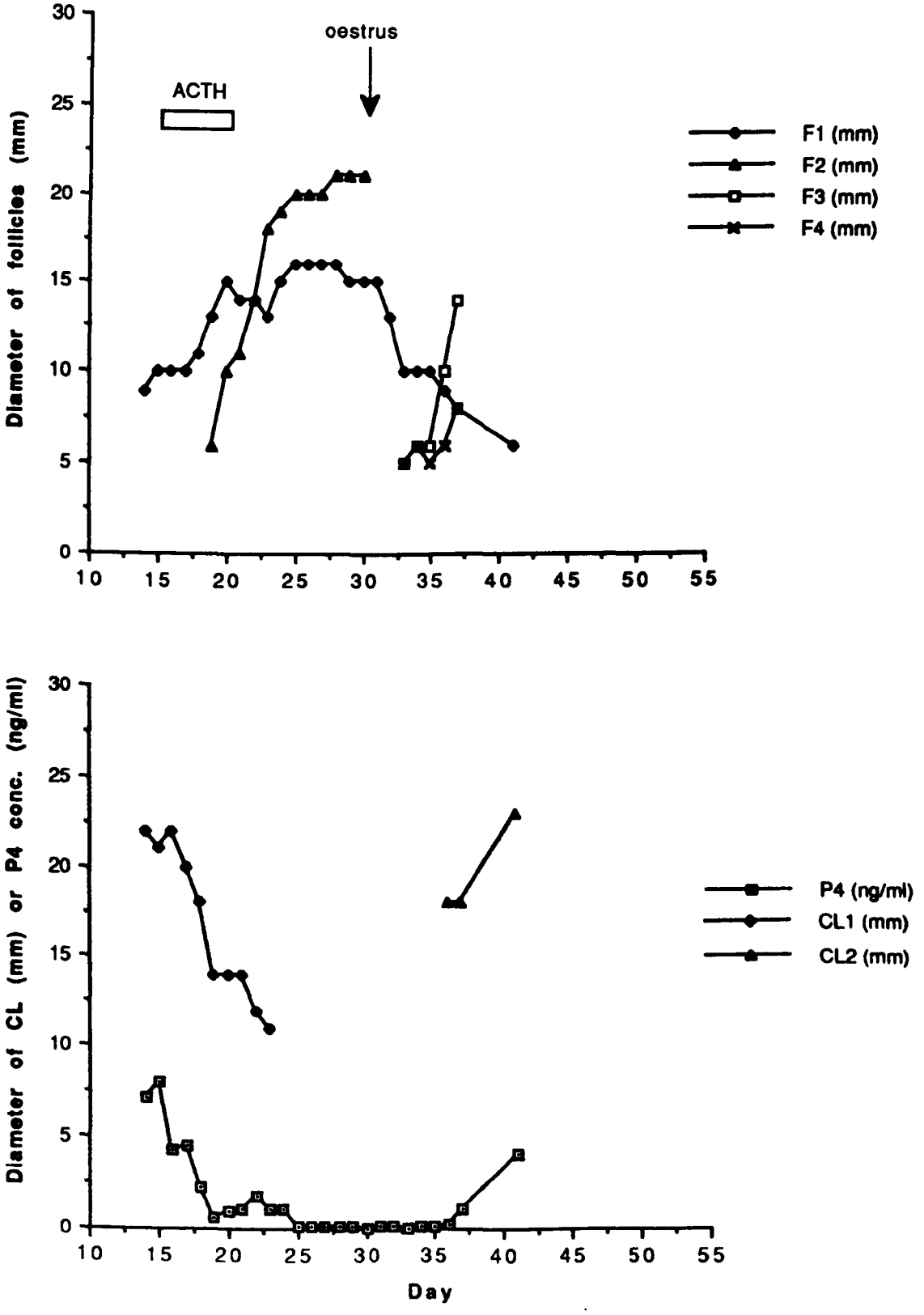


Fig 7.15: Patterns of growth and regression of ovarian structures and progesterone (P4) profile following treatment with ACTH as depicted by horizontal bar (H286)

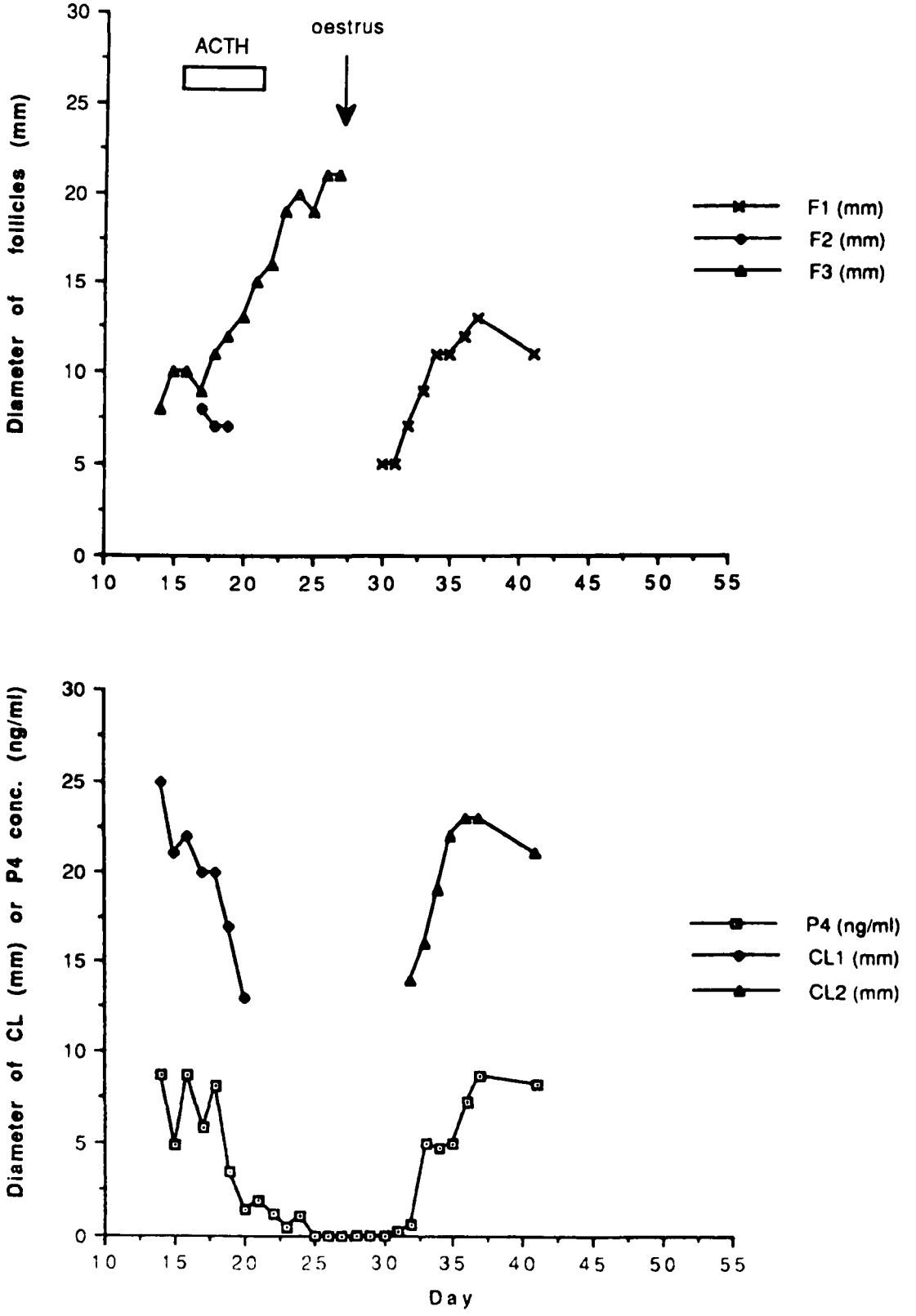


Fig 7.16: Patterns of growth and regression of ovarian structures and progesterone (P4) profile following treatment with ACTH as depicted by horizontal bar (H 638)

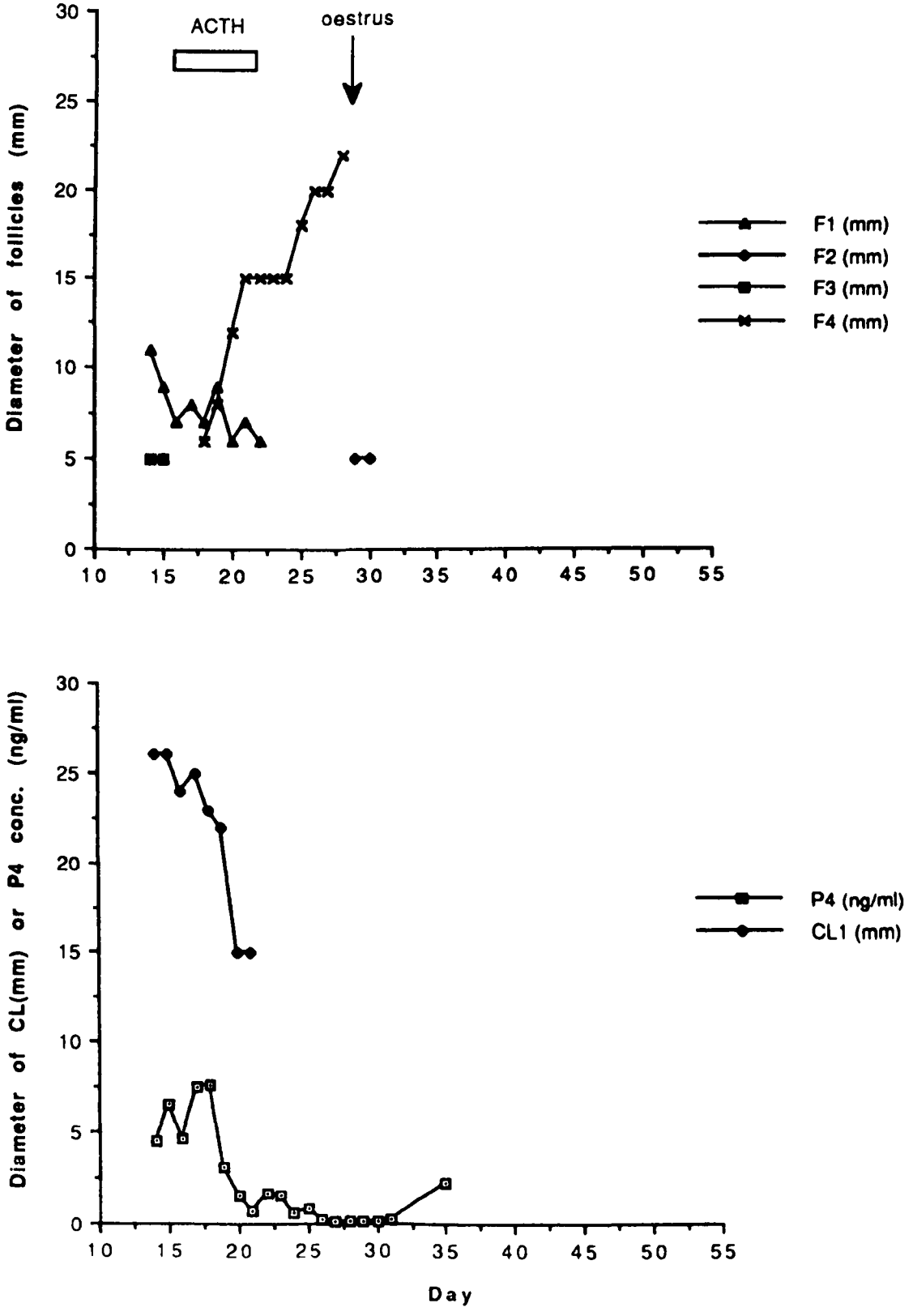


Fig 7.17: Pattern of growth and regression of ovarian structures and progesterone (P4) profile following treatment with ACTH as depicted by horizontal bar (H2283)

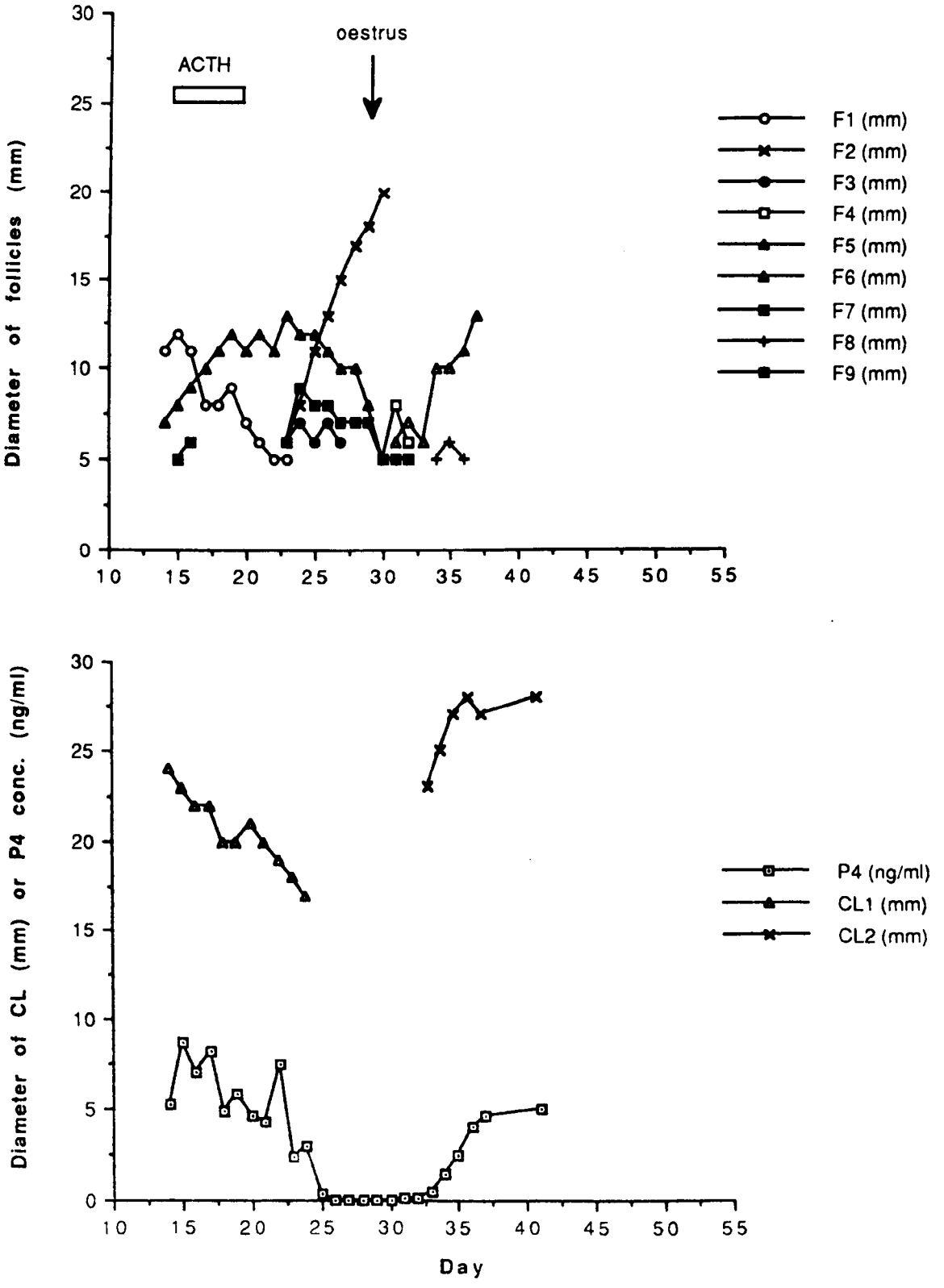


Fig 7.18: Pattern of growth and regression of follicular cyst (FC), other ovarian structures and progesterone profile following treatment with ACTH as depicted by horizontal bar. Horizontal line represents cyst existence (H263)

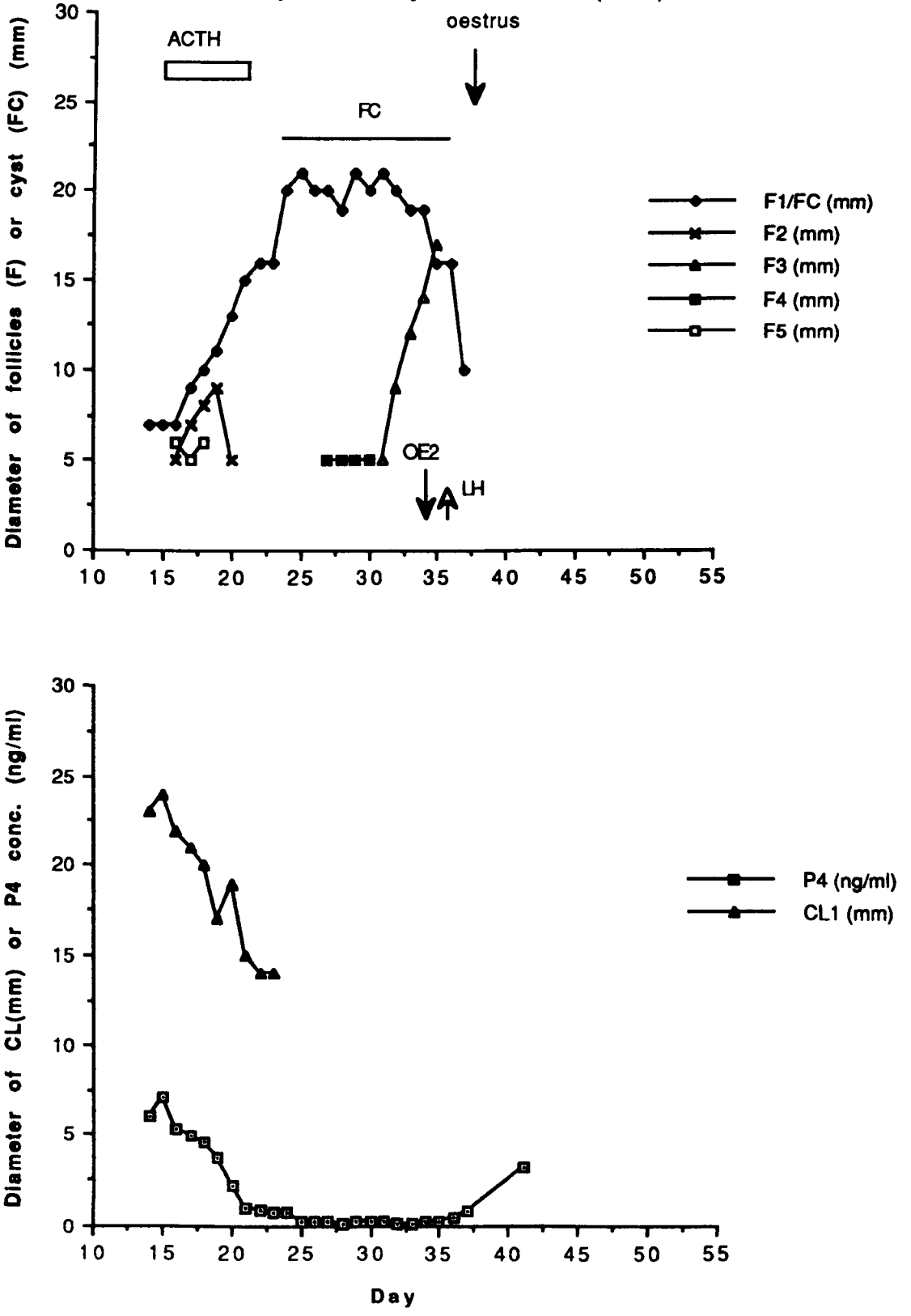


Fig 7.19: Patterns of growth and regression of follicular cyst (FC), other ovarian structures and progesterone (P4) profile after treatment with ACTH as depicted by horizontal bar. Horizontal line represents existence of cyst (H283).

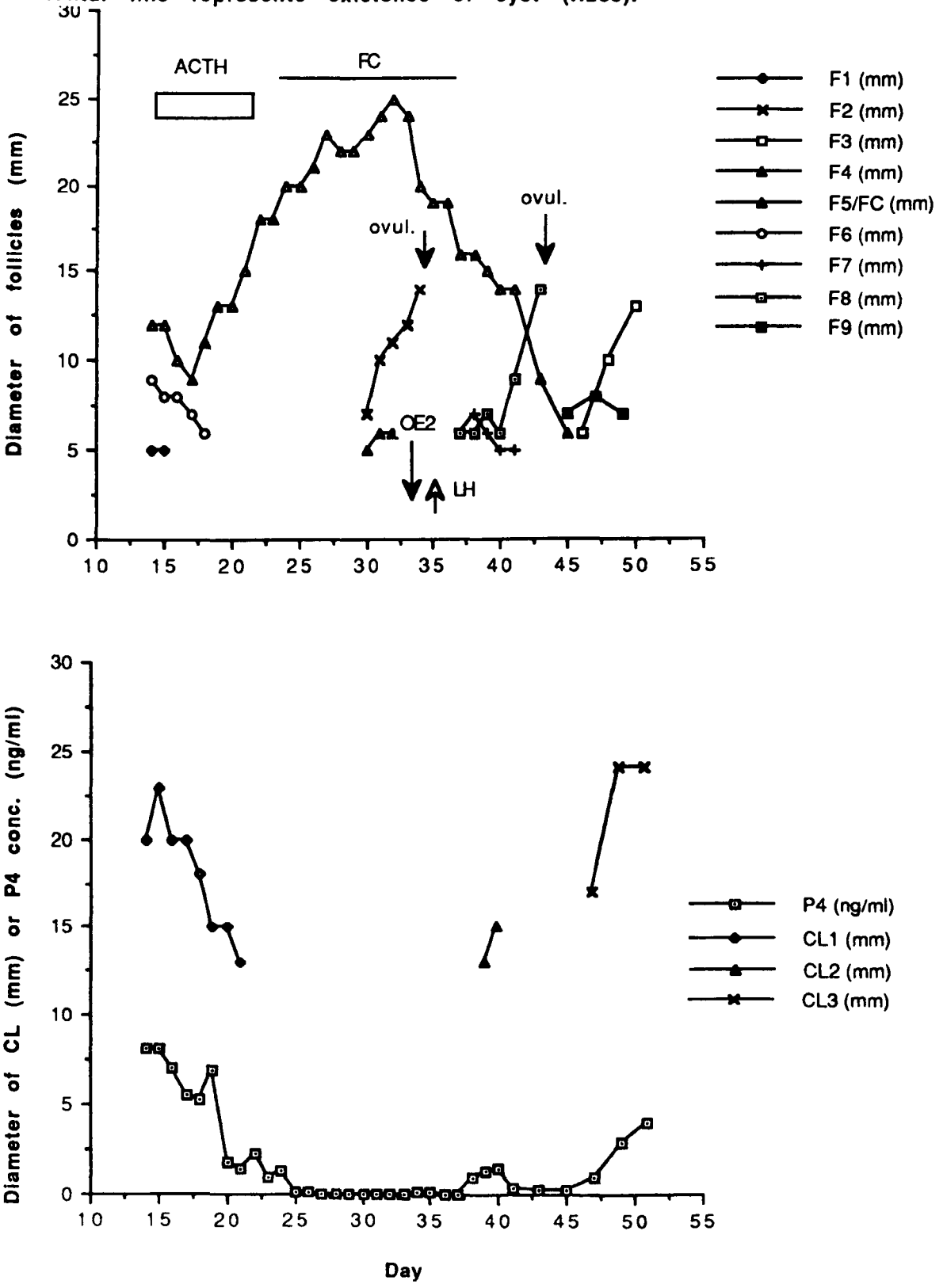


Fig 7.20: Patterns of growth and regression of follicular cyst (FC) and other ovarian structures and progesterone profile following treatment with ACTH as depicted by horizontal bar. Horizontal line represents existence of cyst (H636).

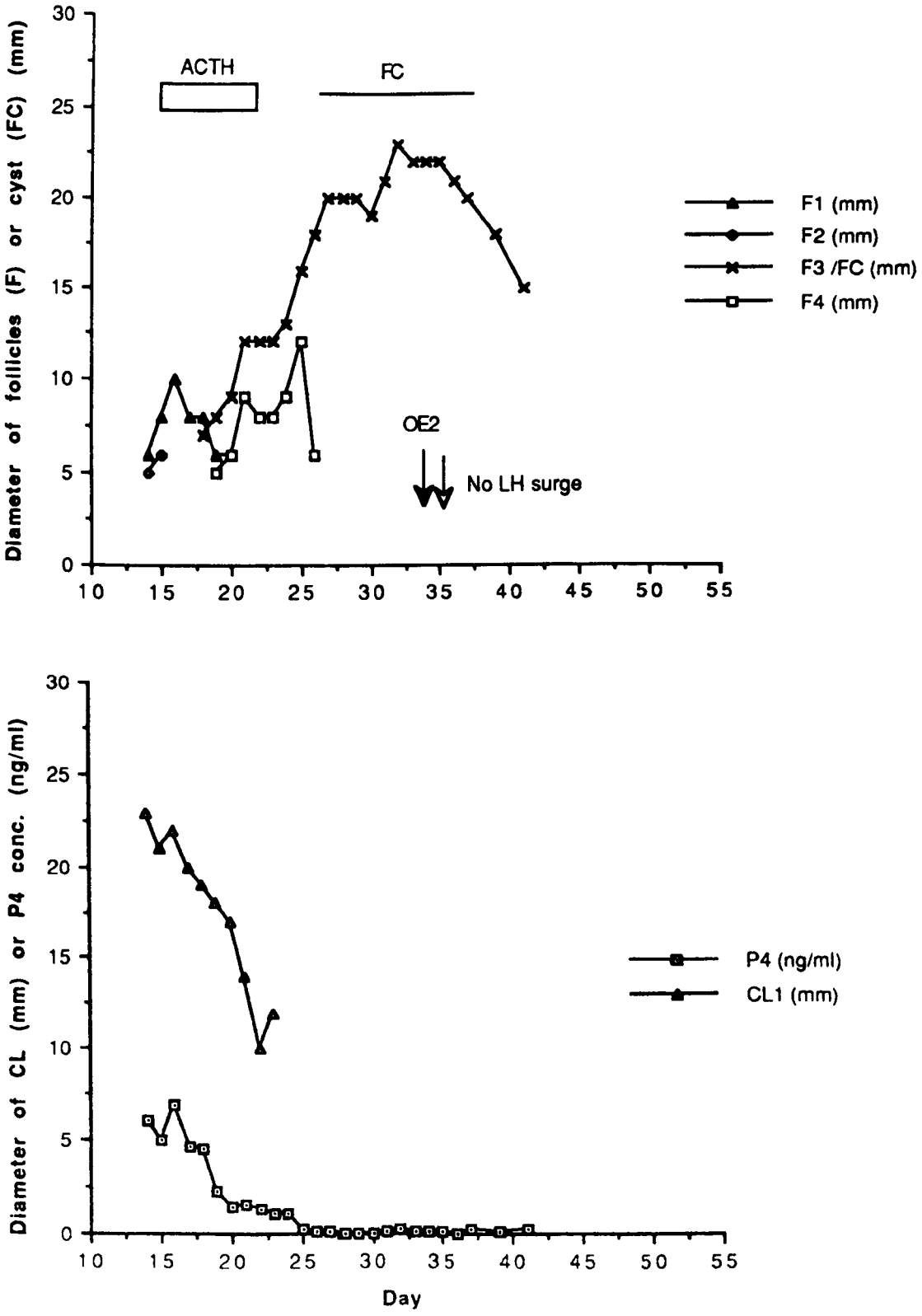
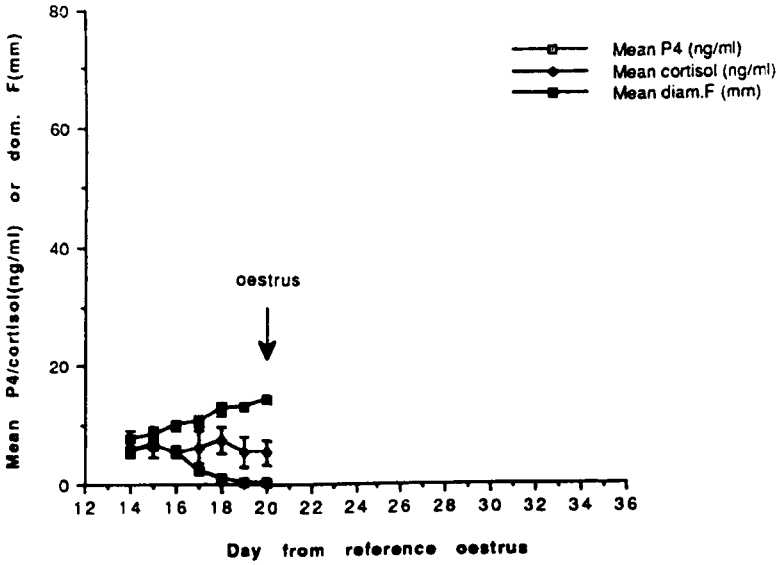
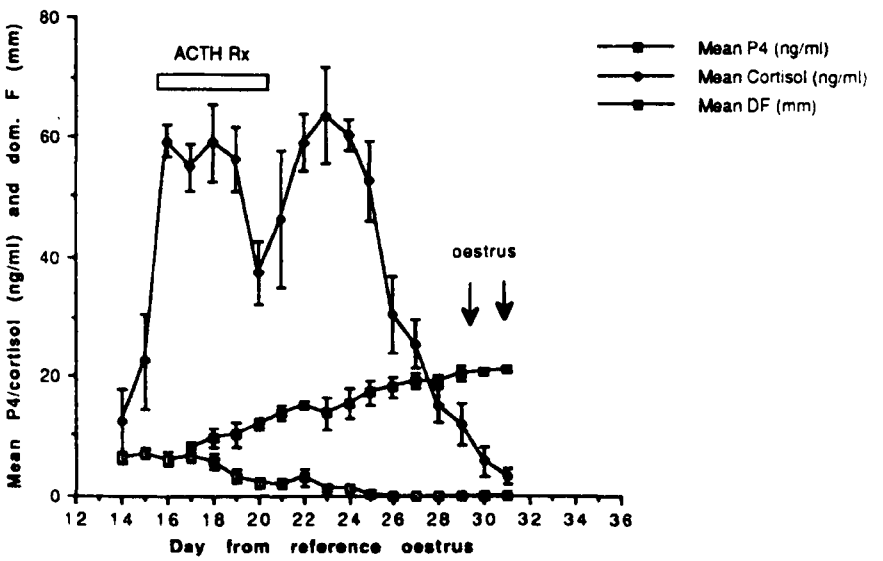


Fig 7.21a: Mean (± S.E.M.) diameter of dominant ovulatory follicle (F) and progesterone (P4) and cortisol concentration during control cycle (day 14-20) (n=6 heifers)



(b): Mean (± S.E.M.) concentration of progesterone (ng/ml), cortisol (ng/ml) and diameter of the dominant follicle (DF) that ovulated in 4 heifers that failed to develop ovarian cysts after treatment with ACTH



(c): Mean (± S.E.M.) concentration of progesterone (ng/ml), cortisol (ng/ml) and diameter of the follicle that became cystic (CF) in 3 heifers after treatment with ACTH

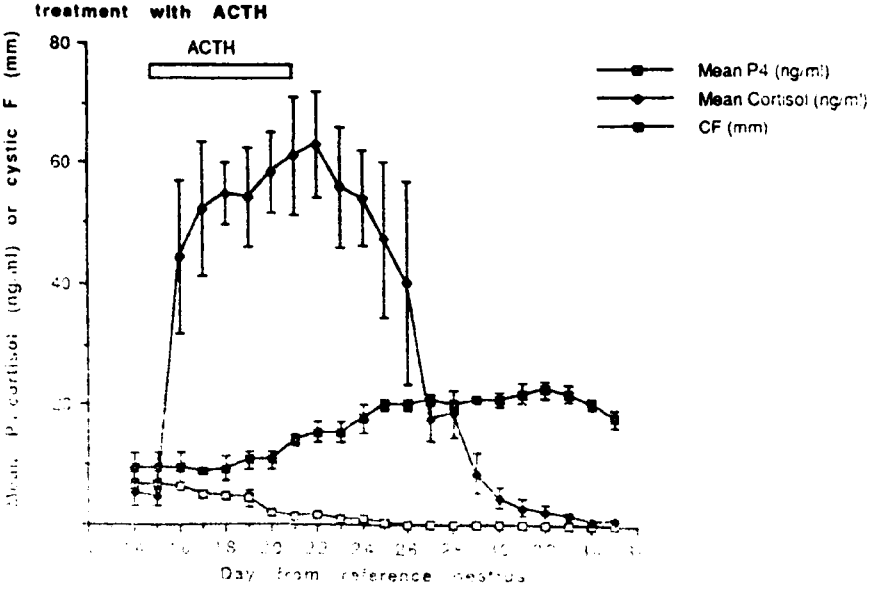


Fig 7.22: Mean (± S.E.M.) cavity diameter of ovulatory and dominant nonovulatory follicles control in 4 heifers with 2 waves/cycle

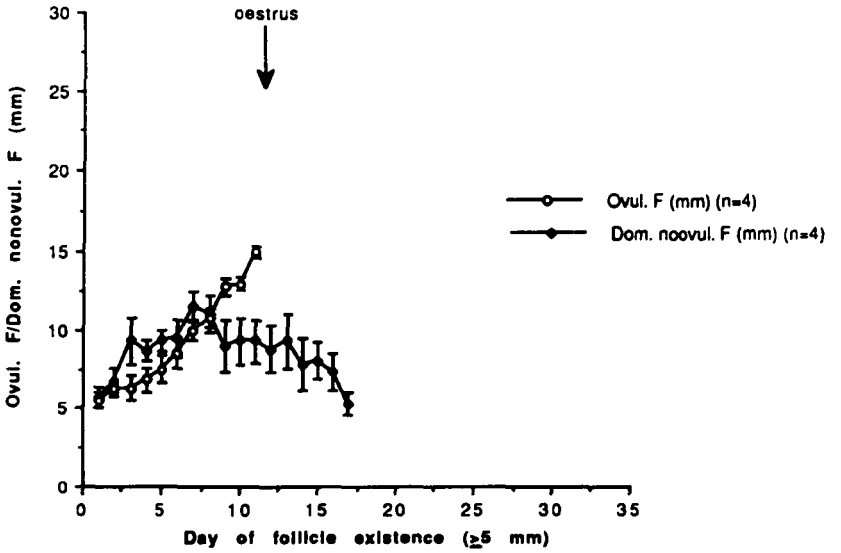


Fig 7.23: Mean (± S.E.M.) cavity diameter of ovulatory follicles (and time of oestrus), dominant nonovulatory and cystic follicles in 8 heifers treated with progesterone/oestradiol

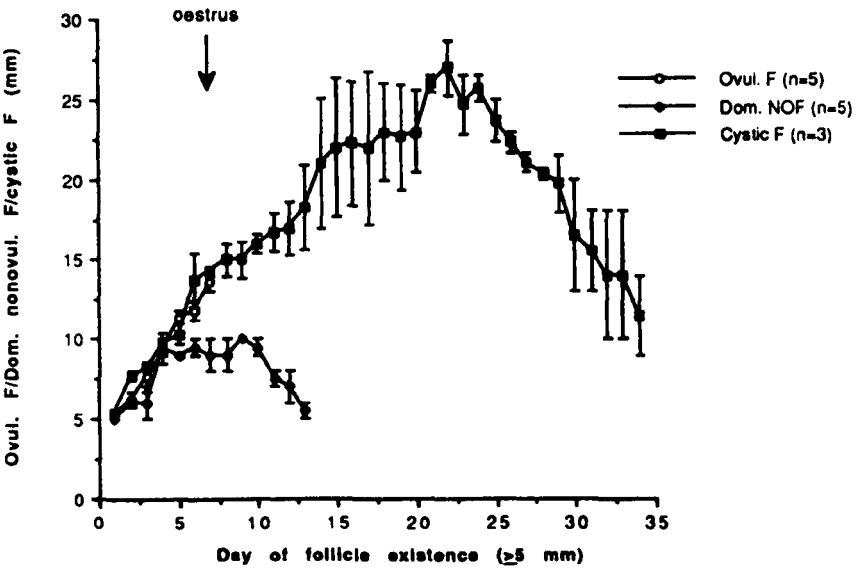
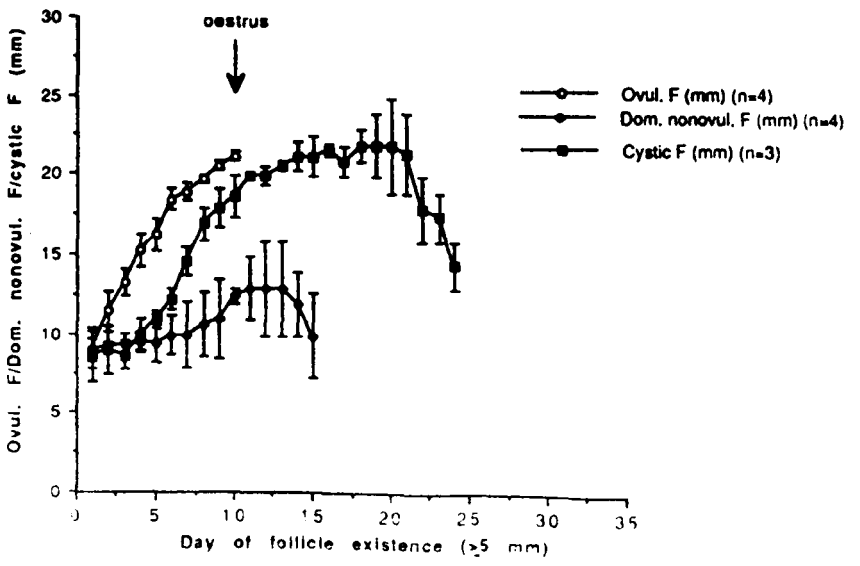


Fig 7.24: Mean (±S.E.M.) cavity diameter of ovulatory follicles (and time of oestrus), dominant nonovulatory and cystic follicles in7 heifers treated with ACTH



CHAPTER EIGHT

GENERAL DISCUSSION AND CONCLUSIONS

The in-vitro study of ovarian structures showed a close relationship between the result of ultrasonography and of ovarian dissection. Ultrasonography was accurate in identifying and measuring normal ovarian structures. The complete agreement between ultrasonography and slicing measurement in identification of mature CL and CL with cavities is similar to the results of *Pierson and Ginther* (1984). The in-vitro study of ovarian structures was useful in learning its ultrasound appearance and in interpretation of the 2-dimensional representation of a 3-dimensional structure. This line of action is recommended as the first step to all those intending to work on bovine reproductive ultrasonography.

During ultrasound and progesterone monitoring of CL through a complete oestrous cycle in 6 postpartum cyclic cows (chapter 4 - study 1), a CL was clearly detectable on ultrasonography by day 5 after ovulation. A high correlation was obtained ($r = 0.85$) between CL diameter and progesterone concentration. However, over the last few days of the cycle, there was a rapid decline in progesterone concentration while the CL diameter decreased only slightly. Thus, a CL during the last few days of the oestrous cycle may be morphologically present but functionally inactive. A recent study (Kamimura *et al.*, 1993) concurred with the present finding.

Both palpation per rectum and ultrasonography had a high sensitivity for identifying mature CL (85% vs 95.7%) in the abattoir study (chapter 4 - study 2). The accuracy of palpation per rectum and ultrasonography for identifying mature CL in the present study is consistent with previous studies (Kähn and Leidl, 1986; Pieterse *et al.*, 1990; Archbald *et al.*, 1992; Robertson *et al.*, 1993).

Ultrasonography had a higher sensitivity in the present study than in previous reports. This may be attributed to differences in types of transducers used and the route of approach.

Only 28% of follicles $\geq$ 10 mm were detected by palpation per rectum whereas ultrasonography identified 76%. This result indicates that palpation per rectum can be a poor method of identifying follicles on ovaries.

In chapter 4 (study 3) palpation per rectum and ultrasonography were compared for the diagnosis and classification of ovarian cysts and compared with plasma progesterone. Of 29 cows presented with cysts by 4 clinicians, only 15 (52%) had cysts (5 follicular; 10 luteal), the remaining 14 cows had normal ovarian structures. Ultrasonography revealed the presence of cysts in 15 cows (6 follicular and 9 luteal), CL with or without cavities in 11 cows and large follicles 12-14 mm in 3 cows. Ultrasound classification was consistent with plasma progesterone in 14/15 (93%) cows :- one luteal cyst was wrongly classified as follicular. Farrin *et al.* (1990) has reported that about 10% of cystic ovaries diagnosed by palpation per rectum had normal ovarian structures when examined by ultrasonography.

Corpora lutea, 4 with and 7 without cavities, accounted for the most misdiagnoses (79%). Farin *et al.* (1990) also reported that a large CL adjacent to one or more large follicles was the most commonly misdiagnosed structure. Other structures which may confound the diagnosis of cysts by palpation per rectum (though not encountered in this study) include cysts near the ovary, small abscesses and ovarian tumors (Youngquist, 1986).

The limitation of a single progesterone measurement for the confirmation of cyst diagnosis by palpation per rectum is also highlighted in the present study. If

only progesterone determination were relied upon for the confirmation of ovarian cyst diagnosis, the 3 cows with large follicles (12 - 14 mm) associated with low progesterone (0.46 $\pm$ 0.05 ng/ml) would have been wrongly "confirmed" as having follicular cysts while the 11 cows with CL (progesterone : 6.15 $\pm$ 0.50 ng/ml) would similarly be wrongly "confirmed" as having luteal cysts. The implication is that failure to diagnose accurately may lead to erroneous conclusions regarding efficacy of treatment. Ultrasonography has proved quite reliable in accurately detecting and categorising ovarian cysts in cattle.

In chapter 5 (study - 1), the spontaneous development of luteal cysts in 2 out of 18 postpartum cows monitored weekly from day 7 after calving until day 42 - 49 suggests that luteal cysts do not necessarily arise from follicular cysts. In a similar study, Carroll *et al.* (1990) observed the spontaneous development of luteal cysts in 8 cows and follicular cysts in 3 cows from a total of 46 cows monitored by weekly ultrasonography from day 14 to 100 after calving.

In seven cows with follicular cysts monitored for 2 weeks, there was lack of luteinization of the cyst wall and progesterone remained continually low. This finding is consistent with the results of Savio *et. al.* (1990) and Sawamukai *et al.* (1991). The lack of correlation (r=0.04) between cyst wall volume and progesterone in cows with follicular cysts is not surprising because follicular cysts tended to be larger (though not significantly different from luteal cysts) and the cyst wall volume substantial, but as a result of lack of luteinization, progesterone remained low. Cyst luteal tissue volume was weakly correlated with plasma progesterone (r=0.54). Luteal tissue volume decreased nonsignificantly 24 h after cloprostenol compared to luteal tissue volume during days 1 - 11 of cyst monitoring and was still visible

on ultrasound (though less homogenous). Progesterone concentration, on the other hand, decreased from 2.78 $\pm$ 0.83 to 0.63 $\pm$ 0.15 ng/ml within 24 h. The slow decline in cyst luteal tissue volume is similar to observations on CL during the last few days of the oestrous cycle (Chapter 4-Study 1; Ribadu *et al.*, 1992). Variability in the volume of luteal tissue in luteal cysts and progesterone concentration may account for the weak correlation.

There were significantly ($P<0.05$) more follicles ($\geq$ 5 mm) in the contralateral ovary than the ovary ipsilateral to the cyst (irrespective of cyst type). This suggests that the presence of a cyst may inhibit the development and maturation of other follicles in the same ovary. More detailed studies are required in this direction. The observation of a wave-like pattern of follicular growth in the ovaries of 16 out of 18 cows indicates that even in the presence of an abnormality (cyst), follicular growth (mostly on the contralateral ovary to the cyst) mimics normal follicular growth pattern. Conclusive statements could not be made because of the limited number of cows and duration of study (2 weeks) which is less than the normal oestrous cycle length in the cow. A wave pattern of follicular growth was not discernible in ovaries of 2 cows (both with follicular cysts).

Response rate to exogenous oestradiol in cows with spontaneous cysts was low (1 in 7 follicular and 3 of 11 luteal). Various response rates (0 - 50%) have been reported previously (Zaied *et al.*, 1981; Dobson and Alam, 1987; Nanda *et al.*, 1991).

In chapter 6, of five cows with follicular cysts, treated with GnRH 10 days after initial diagnosis, two responded by luteinization of cyst wall and three by development of CL from other follicles, accompanied by an elevated progesterone

concentration by day 7 after treatment. Other changes noted during weekly ultrasonography included clouding of the uniformly nonechogenic antrum of cysts(as observed in spontaneous luteal cysts), reduction in cyst size or cyst resolution. In the 2 cows that responded by luteinization, an increase in the cyst wall thickness at the expense of cyst cavity was evident.

A weak correlation was reported between assessment of luteinization by palpation per rectum after treatment, and milk or plasma progesterone (Garverick *et al.*, 1976; Nakao *et al.*, 1983). Incorrect assessment of luteinization of follicular cysts has been a problem (Whitmore *et al.*, 1979; Nakao and Kawata, 1980) and has led to unnecessary treatment in many cows (Nakao *et al.*, 1983). Ultrasonography, by visualising the ovarian structures, was able to assess luteinization and/or development of CL accurately following treatment in the present study. An increase in plasma progesterone concurred with ultrasound findings of luteinization or CL formation.

In chapter 7, daily ultrasound and progesterone monitoring of control oestrous cycles in 6 heifers revealed 2 waves of follicular growth in 4 heifers and 3 waves of follicular growth in 2 heifers. The dominant follicle of the second (n=4) and third (n=2) wave ovulated whereas the dominant follicle of the preceding wave(s) underwent atresia.

Ovarian cysts were successfully induced in 3/8 (38%) heifers using progesterone/oestradiol treatment and 3/7 (43%) heifers using ACTH treatment, both during the late luteal phases of the oestrous cycle. In previous studies, ovarian cysts were induced in 8/15 (53%) cows using progesterone/oestradiol (Cook *et al.*,

1991) and in 2/5 (40%) ACTH-treated cows. In contrast to previous studies, cyst development was monitored by ultrasonography in the present study.

A static phase was noted in all heifers treated with progesterone/oestradiol. The static phase was shorter (3-16 days) than the 29-41 days reported by Cook *et al.* (1990). No static phase was observed in ACTH-treated heifers or during the control oestrous cycles. It is of interest that a diversity of cysts (1 follicular; 2 luteal) resulted from the same progesterone/oestradiol treatment. Ovarian cysts in 3 heifers that resulted from ACTH treatment were, however, all follicular. Both treatments led to alteration of oestrous cycles with extension of interovulatory intervals, irrespective of whether ovulation or cyst formation occurred subsequently. From the mean diameters of dominant ovulatory follicles (control and steroid-treated, noncystic), dominant nonovulatory (control and steroid-treated noncystic) and dominant follicles that became cystic (treated cystic heifers) it is apparent that the "decision" whether a dominant follicle was going to be nonovulatory was made early after emergence of the follicle. In contrast, dominant follicles that subsequently ovulated or became cystic continued to grow until a "decision" for ovulation or persistence as a cyst was made a few days later. What factors determine whether ovulation or cyst formation should occur after hormone treatment is not clear. The initial growing phase of follicles into cysts seen in experimentally-induced cysts was not observed in spontaneous cysts probably because spontaneous cysts were only detected at a later stage.

Short luteal phases were noted in 2 heifers in the progesterone/oestradiol treated heifers and in 1 heifer in the ACTH-treated group. This observation is a

significant incidental finding. These treatments may provide models for detailed studies into the phenomenon of short luteal phases in cattle.

Overall, 3 of 6 heifers with steroid-induced cysts responded by producing an LH surge to exogenous oestradiol. It appears that the response of cattle with experimentally induced cysts is also variable, similar to spontaneous cysts (Zaied *et al.*, 1981; Dobson and Alam, 1987; Dobson and Nanda, 1992). Refsal *et al.* (1987) reported an LH surge in only one out of 4 cows with experimentally induced cysts. More such work is required with induced cysts if the aetiology of spontaneously occurring cysts is to be determined.

The lack of luteinization in 7 spontaneous follicular cysts monitored for 2 weeks and the spontaneous formation of luteal cysts in 2 cows monitored during the early postpartum period (chapter 5) coupled with the formation of both follicular and luteal cysts after steroid treatment of heifers (chapter 7) all seem to indicate that cysts may belong to two or three different populations rather than arising from one population. However, it has not been proven beyond doubt that some follicular cysts may transform into luteal cysts with time while others remain follicular until regression occurs. It would be interesting to know exactly how growth rates of follicles are determined (pulse rate of LH; availability of FSH) and whether this determines the eventual outcome of follicular activity i.e steroidogenesis, atresia, dominant ovulatory or nonovulatory follicle, luteal or follicular cyst.

This thesis has provided additional and new information on the subject of bovine ovarian ultrasonography and raised questions that require further detailed investigation. Although the significance and limitations of ultrasonography and hormone measurement have been highlighted in terms of clinical diagnosis, exciting

new revelations have been made concerning growth rates of normal and abnormal follicles. This has set the stage for future detailed studies on the pathology of ruminant ovaries.

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APPENDIX I

APPENDIX IA (ovarian cyst diagnosis by palpation and ultrasonography)

S/No	Cow No	Lactation	Palpation	Ultrasonography	P_4 (ng/ml)
1	60	3	Luteal cyst (LO)	LO: Luteal cyst ID= 30 mm OD= 38 mm RO: F= 10mm	2.50
2	174	4	Luteal cyst (LO)	LO: Luteal cyst ID= 28 mm OD= 34 mm RO: F=<5 mm	5.24
3	73	3	Luteal cyst (RO)	LO: F=20 mm RO: Luteal cyst ID= 33 mm OD= 44 mm	5.40
4	3	4	Luteal cyst (LO)	LO: CL= 21x25 mm F= 21 mm RO: F= 10 mm	6.9
5	82	3	Luteal cyst (LO)	LO: CL= 28x22 mm RO: F=<5 mm	4.61
6	275	7	Follicular cyst (RO)	LO: F=<5 mm RO: Follicular cyst ID= 29 mm OD= 33 mm	0.55
7	240	4	Luteal cyst (RO)	LO: F=15 mm RO: Luteal cyst ID= 22 mm OD= 31 mm	3.70
8	743	2	Luteal cyst (LO)	LO: CL= 33x14 mm RO: F=<5 mm	3.29
9	182	5	Luteal cyst (RO)	LO: F=<5 mm RO: CL= 24x27 mm	6.90
10	799	5	Follicular cyst (RO)	LO: F=7 mm RO: Follicular cyst ID= 42 mm OD= 48 mm	0.17
11	40	2	Luteal cyst (RO)	LO: F=<5 mm RO: Luteal cyst ID= 27 mm OD= 33 mm	5.16
12	190	3	Luteal cyst (RO)	LO: CL= 19x20 mm CL= 16x16 mm F= 10 mm RO: F=<5 mm	7.00

13	22	3	Luteal cyst (RO)	LO: F= 15 mm F= 7 mm RO: Luteal cyst ID= 30 mm OD= 41 mm	4.65
14	37	4	Luteal cyst (RO)	LO: Luteal cyst ID= 20 mm OD= 40 mm RO: F=8 mm F=6 mm	3.50
15	72	7	Follicular cyst (RO)	LO: F= 12 mm F= 10 mm RO: Follicular cyst ID= 35 mm OD= 40 mm	0.36
16	23	5	Follicular cyst (RO)	LO: F=<5 mm RO: F= 12 mm	0.50
17	77	4	Luteal cyst (RO)	LO: F= 10 mm RO: Luteal cyst ID= 30 mm OD= 36 mm	1.48
18	37	4	Follicular cyst (RO)	LO: F= <5 mm RO: F= 14 mm	0.50
19	53	3	Follicular cyst (RO)	LO: F=<5 mm RO: F= 13 mm F= 11 mm	0.36
20	S470	5	Luteal cyst (RO)	LO: F= 10 mm F= 7 mm RO: Luteal cyst ID= 25 mm OD= 32 mm	2.23
21	33	5	Luteal cyst (LO)	LO: CL= 30x22 mm RO: F=12 mm F= 9 mm	7.00
22	63	3	Luteal cyst (LO)	LO: Follicular cyst ID= 37 mm OD= 43 mm RO: F=7 mm F=6 mm	1.52
23	68	6	Luteal cyst (LO)	LO: CL= 28x24 mm RO: F=<5 mm	6.90
24	176	5	Luteal cyst (LO)	LO: CL= 25x29 mm RO: F= 15 mm	3.60
25	35	9	Follicular cyst (RO)	LO: 7 mm RO: Follicular cyst ID= 24 mm OD= 26 mm	0.90

26	175	4	Luteal cyst (RO)	LO: F= < 5 mm RO: CL= 24x15 mm F= 20 mm	7.00
27	29	3	Luteal cyst (LO)	LO: CL= 27x26 mm RO: F= <5 mm	6.90
28	844	4	Follicular cyst (LO)	LO: Follicular cyst ID= 46 mm OD= 49 mm	0.08
29	31	5	Luteal cyst (RO)	LO: F= 13 mm RO: CL= 25x19 mm F= 14 mm	7.00

APPENDIX IB (Dynamics of spontaneous cysts)

Cow 174

Day	Inner diameter (mm)	Outer diameter (mm)	Cyst wall vol. (x1000 mm^3)	Progesterone (ng/ml)
1	28	34	11.49	5.24
3	23	32	10.78	3.85
5	22	31	10.02	1.80
7	19	28	6.83	1.42
9	19	27	6.71	1.60
11	20	28	7.30	1.40

Cow 60

Day	Inner diameter (mm)	Outer diameter (mm)	Cyst wall vol. (x1000 mm^3)	Progesterone (ng/ml)
1	30	38	4.59	2.50
3	28	36	12.93	3.20
5	28	37	15.02	3.30
7	27	38	18.42	3.50
9	28	38	17.23	3.40
11	28	39	19.55	3.60
12	24	34	11.67	0.90

Cow 73

Day	Inner diameter (mm)	Outer diameter (mm)	Cyst wall vol. (x1000 mm^3)	Progesterone (ng/ml)
1	33	44	25.78	5.40
3	33	42	19.97	4.10
5	34	43	21.04	4.30
7	34	41	15.50	1.30
9	36	42	14.53	0.20
11	35	41	13.63	0.20
12	31	38	13.13	0.20

Cow 240

Day	Inner diameter (mm)	Outer diameter (mm)	Cyst wall vol. (x1000 mm^3)	Progesterone (ng/ml)
1	22	31	10.02	3.70
3	26	33	9.35	6.50
5	25	34	11.67	4.00
7	24	34	12.62	2.30
9	18	31	12.72	0.50
11	13	22	4.52	0.45
12	13	22	4.52	0.44

Cow 40

Day	Inner diameter (mm)	Outer diameter (mm)	Cyst wall vol. (x1000 mm^3)	Progesterone (ng/ml)
1	27	33	8.50	5.16
3	28	34	8.36	6.58
5	30	34	6.44	9.02
7	28	35	10.95	1.98
9	29	33	6.69	0.66
11	27	34	9.93	0.06
12	27	33	8.53	0.06

Cow 22

Day	Inner diameter (mm)	Outer diameter (mm)	Cyst wall vol. (x1000 mm^3)	Progesterone (ng/ml)
1	30	41	21.94	4.65
3	30	40	20.05	3.36
5	27	38	17.86	3.30
7	25	32	8.18	2.50
9	15	25	6.41	2.40
11	8	15	1.50	2.28
12	7	14	0.64	0.51

Cow 77

Day	Inner diameter (mm)	Outer diameter (mm)	Cyst wall vol. (mm)	Progesterone (ng/ml)
1	30	36	8.56	1.28
3	36	44	19.67	4.43
5	29	36	11.66	3.61
7	16	31	12.71	3.07
9	17	29	9.76	4.13
11	18	29	9.96	3.70
12	16	28	8.74	0.42

Cow S470

Day	Inner diameter (mm)	Outer diameter (mm)	Cyst wall vol. (x1000 mm^3)	Progesterone (ng/ml)
1	25	32	8.18	2.23
3	25	31	7.89	3.95
5	26	30	4.24	6.54
7	24	28	4.09	12.50
9	20	25	3.99	12.06
11	20	27	5.86	13.70
12	21	28	6.03	0.59

Cow 63

Day	Inner diameter (mm)	Outer diameter (mm)	Cyst wall vol. (x1000 mm^3)	Progesterone (ng/ml)
1	37	42	12.26	1.52
3	39	45	16.65	4.66
5	37	43	15.10	7.21
7	37	44	18.07	7.10
9	35	44	22.14	7.19
11	36	42	14.35	8.12
12	36	42	14.35	1.14

Cow 42

Day	Inner diameter (mm)	Outer diameter (mm)	Cyst wall vol. (x1000 mm^3)	Progesterone (ng/ml)
1	27	35	12.10	2.05
3	31	38	13.13	2.31
5	29	38	15.96	5.88
7	32	41	18.92	5.72
9	29	38	15.96	6.82
11	25	34	12.39	3.66
12	20	26	5.01	0.42

Cow 116

Day	Inner diameter (mm)	Outer diameter (mm)	Cyst wall vol. (x1000 mm^3)	Progesterone (ng/ml)
1	24	34	13.34	9.46
3	25	36	16.24	9.38
5	22	31	10.02	9.10
7	24	33	11.57	0.51
9	24	33	11.57	0.47
11	20	27	6.11	0.10
12	18	26	6.15	0.16

Cow 275

Day	Inner diameter (mm)	Outer diameter (mm)	Cyst wall vol. (x1000 mm^3)	Progesterone (ng/ml)
1	29	33	6.04	0.55
3	30	35	8.31	0.58
5	25	29	4.58	0.56
7	24	28	4.26	0.36
9	20	25	3.99	0.38
11	19	24	3.64	0.48
12	20	25	3.99	0.42

Cow 799

Day	Inner diameter (mm)	Outer diameter (mm)	Cyst wall vol. (x1000 mm^3)	Progesterone (ng/ml)
1	42	48	19.11	0.17
3	38	48	29.16	0.29
5	45	50	17.73	0.13
7	41	50	29.35	0.32
9	47	52	19.25	0.31
11	46	52	22.64	0.29
12	48	53	20.03	0.19

Cow 72

Day	Inner diameter (mm)	Outer diameter (mm)	Cyst wall vol. (x1000 mm^3)	Progesterone (ng/ml)
1	35	40	11.05	0.36
3	35	38	6.28	0.17
5	32	37	9.36	0.20
7	33	37	7.70	0.31
9	32	37	9.36	0.22
11	31	35	6.85	0.23
12	29	32	4.39	0.28

Cow 35

Day	Inner diameter (mm)	Outer diameter (mm)	Cyst wall vol. (x1000 mm^3)	Progesterone (ng/ml)
1	24	26	1.97	0.90
3	26	29	3.56	0.96
5	30	33	4.68	0.48
7	32	36	7.27	0.50
9	32	36	7.27	0.60
11	34	38	8.15	0.41
12	36	39	6.62	0.33

Cow 844

Day	Inner diameter (mm)	Outer diameter (mm)	Cyst wall vol. (x1000 mm^3)	Progesterone (ng/ml)
1	46	49	10.63	0.08
3	46	51	18.48	0.08
5	44	50	20.84	0.12
7	46	52	22.64	0.08
9	35	43	19.17	0.04
11	36	43	17.19	0.09
12	33	41	17.26	0.04

Cow 145

Day	Inner diameter (mm)	Outer diameter (mm)	Cyst wall vol. (x1000 mm^3)	Progesterone (ng/ml)
1	45	50	17.73	0.13
3	41	50	29.35	0.34
5	47	52	19.25	0.31
7	46	52	22.64	0.29
9	48	53	20.03	0.20
11	42	48	19.11	0.17
12	38	48	29.16	0.29

Cows 603

Day	Inner diameter (mm)	Outer diameter (mm)	Cyst wall vol. (x1000 mm^3)	Progesterone (ng/ml)
1	32	36	7.27	0.60
3	34	38	8.15	0.45
5	36	39	6.62	0.36
7	35	40	11.05	0.35
9	35	38	6.28	0.18
11	32	37	9.36	0.20
12	33	37	7.70	0.31

APPENDIX IC

(Follicular dynamics in the presence of ovarian cysts)

Cow 275

Day	F1 (mm)	F2 (mm)	F3 (mm)
1	-	-	-
3	6	6	-
5	6	8	9
7	9	8	10
9	9	-	14
11	5	-	20
12	-	-	18

Cow 799

Day (mm)	F1 (mm)	F2 (mm)	F3 (mm)
1	7	-	-
3	-	-	-
5	-	-	-
7	-	-	-
9	6	-	-
11	6	-	-
12	7	5	-

Cow 72

Day	F1 (mm)	F2 (mm)	F3 (mm)
1	12	10	-
3	18	8	7
5	19	8	9
7	26	5	6
9	28	-	-
11	30	-	-
12	32	-	-

Cow 35

Day	F1 (mm)	F2 (mm)	F3 (mm)
1	7	11	-
3	8	6	-
5	7	-	-
7	6	5	-
9	7	-	-
11	6	-	-
12	7	5	-

Cow 844

Day	F1 (mm)	F2 (mm)	F3 (mm)
1	6	5	-
3	11	-	-
5	16	-	8
7	18	-	13
9	22	-	18
11	22	-	19
12	22	-	22

Cow 603

Day	F1 (mm)	F2 (mm)	F3 (mm)
1	16	9	6
3	18	7	6
5	17	9	-
7	14	11	-
9	15	12	-
11	14	12	-
12	12	16	-

Cow 174

Day	F1 (mm)	F2 (mm)	F3 (mm)
1	-	-	-
3	6	-	-
5	5	-	-
7	7	-	-
9	8	-	-
11	10	-	-
12	11	-	-

cow 60

Day	F1 (mm)	F2 (mm)	F3 (mm)
1	10	-	-
3	9	8	-
5	7	7	-
7	10	9	-
9	11	10	-
11	11	9	-
12	12	10	-

Cow 73

Day	F1 (mm)	F2 (mm)	F3 (mm)
1	20	-	-
3	22	-	-
5	24	-	-
7	24	5	8
9	25	7	13
11	24	5	12
12	25	6	13

Cow 240

Day	F1 (mm)	F2 (mm)	F3 (mm)
1	16	15	-
3	17	14	-
5	15	14	8
7	14	14	13
9	10	18	11
11	10	21	15
12	8	18	12

Cow 40

Day	F1 (mm)	F2 (mm)	F3 (mm)
1	-	-	-
3	-	-	-
5	9	-	-
7	8	7	-
9	12	7	-
11	14	6	-
12	14	-	-

Cow 22

Day	F1 (mm)	F2 (mm)	F3 (mm)
1	15	6	6
3	13	10	7
5	14	11	-
7	12	9	-
9	7	5	-
11	9	8	-
12	7	12	-

Cow 77

Day	F1 (mm)	F2 (mm)	F3 (mm)	
1	10	-	-	
3	13	-	-	
5	6	-	-	
7	7	8	-	
9	5	10	-	
11	6	13	-	
12	8	15	-	

Cow S470

Day	F1 (mm)	F2 (mm)	F3 (mm)
1	10	7	6
3	12	7	-
5	13	9	5
7	11	8	-
9	7	7	8
11	8	5	12
12	9	7	11

Cow 63

Day	F1 (mm)	F2 (mm)	F3 (mm)	
1	7	6	-	
3	8	6	-	
5	6	-	-	
7	6	-	-	
9	9	5	-	
11	10	6	-	
12	11	7	-	

Cow 42

Day	F1 (mm)	F2 (mm)	F3 (mm)
1	13	-	-
3	13	8	5
5	14	13	9
7	14	12	-
9	16	14	-
11	16	10	5
12	14	7	-

Cow 116

Day	F1 (mm)	F2 (mm)	F3 (mm)	
1	11	9	5	
3	10	8	-	
5	8	8	-	
7	6	6	5	
9	8	7	-	
11	10	6	-	
12	10	-	-	

APPENDIX ID

LH data of control cows (225, 134, 293, 121,46 & 146) and cows with ovarian cysts at the time of oestradiol injection (0 h) and 18, 24 h later.

Cow No	Time (h)	LH (ng/ml)		Cow No	Time (h)	LH (ng/ml)
225	0	2.78		275	24	1.67
	18	1.09		799	0	1.41
	24	4.64			18	2.80
134	0	4.63			24	2.24
	18	31.42		73	0	5.20
	24	5.83			18	5.65
293	0	8.38			24	15.90
	18	35.70		603	0	9.27
	24	1.87			18	3.32
146	0	2.46			24	8.57
	18	45.74		145	0	14.68
	24	1.68			18	6.37
121	0	2.38			24	5.43
	18	25.72		42	0	3.97
	24	0.46			18	2.80
46	0	2.27			24	3.05
	18	3.32		116	0	1.33
	24	26.79			18	3.55
35	0	2.80			24	2.53
	18	2.15		S470	0	0.84
	24	1.74			18	1.16
240	0	2.85			24	2.11
	18	5.78		63	0	1.72
	24	1.58			18	2.69
22	0	2.40			24	12.71
	18	1.21		77	0	3.08
	24	0.91			18	3.31
275	0	1.74			24	26.31
	18	1.87		844	0	3.95

844	18	3.75		40	24	6.14
	24	12.25		174	0	0.41
72	0	2.26			18	1.04
	18	4.28			24	1.01
	24	3.70		60	0	0.84
40	0	19.48			18	1.30
	18	2.65			24	1.95

APPENDIX II

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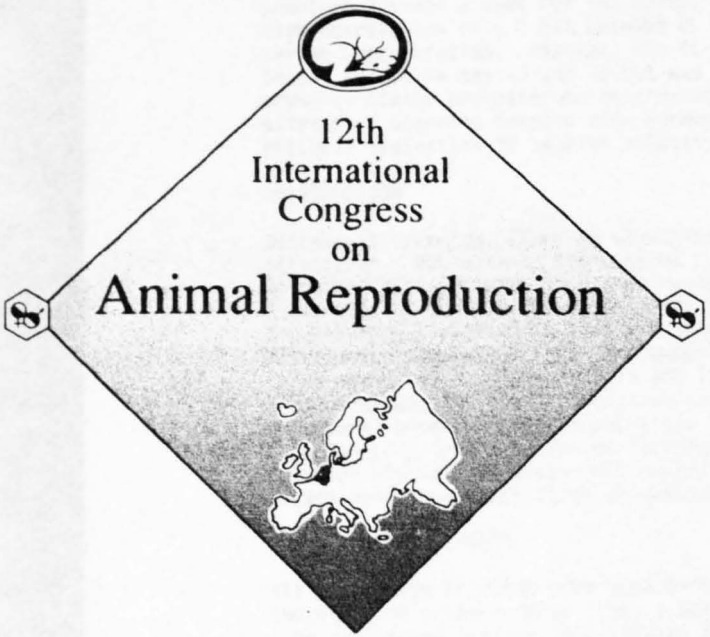

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ULTRASONIC EVALUATION OF CORPORA LUTEA AND PLASMA PROGESTERONE PROFILE DURING THE OESTROUS CYCLE IN POSTPARTUM COWS

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SUMMARY

The ovaries of 6 lactating cyclic Friesian dairy cows were scanned (for CL diameter), and blood samples were obtained via tail venepuncture 3 times a week for one month. On most days, there was a high correlation ($r = 0.85$) between CL diameter and plasma progesterone concentration. However, the CL diameter remained at mid-luteal values on day -3 and -2 but was functionally inactive, as shown by plasma progesterone determination. In conclusion, ultrasonic scanning coupled with hormone measurements gave the most reliable evaluation of ovarian activity.

INTRODUCTION

Ultrasonic scanning offers an objective assessment of ovarian structures. Decrease in the size of corpora lutea (CL) in heifers coincided with decrease in plasma concentration of progesterone (1). A correlation coefficient of 0.68 was obtained between RIA (milk) progesterone concentration and ultrasonographic luteal diameter (2). Ultrasonic assessment of CL was compared with progesterone profiles in non-pregnant Holstein heifers and it was concluded that ultrasonography was a viable alternative to laboratory determination of peripheral progesterone concentration for the assessment of CL function (3). The objective of the present study was to determine the relationship between ultrasonic determination of corpora lutea and plasma progesterone profiles in postpartum cyclic cows.

MATERIALS AND METHODS

Six postpartum Friesian cows aged 4-10 years (weighing between 420 - 590 kg) were scanned three times a week for one month using a real-time ultrasound scanner (SSD 210DXII Aloka, BCF Technology Ltd., Livingstone, Scotland) equipped with a 7.5 MHz linear array rectal transducer. Faeces were evacuated from the rectum before examination. Each ovary was scanned in several planes in order to identify a CL. Maximum diameter of the CL was measured using the in-built electronic calipers after freezing the image on the screen. Blood samples were collected after each examination by tail venepuncture. Oestrus detection was carried out three times a day by observation of mounting behaviour. Plasma progesterone concentration was determined by radioimmunoassay (4). The intra and inter-assay coefficients of variation were 2.74% and 13.80% respectively and the sensitivity of the assay was 0.05 ng/ml. CL diameter and progesterone concentration were correlated using Pearsons correlation (5).

RESULTS AND DISCUSSION

The mean length of the oestrous cycle in the 6 cows was 21.16 + 0.40 days. The relationship throughout the oestrous cycle between CL diameter and plasma progesterone concentration is given in Fig. 1. A corpus luteum was only detectable by ultrasound from day 4 onwards. The appearance of a CL was that of a granular, echogenic structure with well defined borders. The appearance of a cavity at the centre of the CL was observed in one cow (cow 4). The cavity appeared as a non-echogenic (dark) area surrounded by a rim of echogenic luteal tissue.

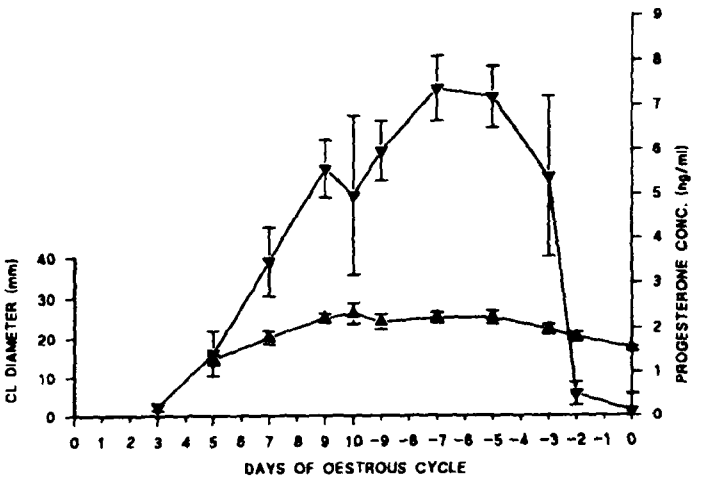

Fig 1. Ultrasonographic determination of CL diameter (▲) and plasma progesterone concentration (▼) in 6 cows (mean +s.e.m.)

The high correlation (r = 0.85) between CL diameter and progesterone concentration was consistent with a previous study (6). The mean peak plasma progesterone concentration of 7.4 + 0.71 ng/ml was similar to previous reports (4,7). In the present study the CL size ranged from 1.45 + 0.13 cm on day 5 to a maximum value of 2.6 + 0.25 cm on day 10 of the cycle, similar to findings in non-pregnant cattle (8). The ultrasonic detection of CL with cavities have been reported previously (9). Progesterone concentration for cow 4 was similar to that of other cows without cavities in the CL confirming previous studies (1,10).

Although there was a high correlation (r = 0.85) between CL diameter

and progesterone profile, our results do not support the conclusion (3) that ultrasonic determination of CL could be a viable alternative to peripheral progesterone determination. In the present study over the last few days of the cycle there was a rapid decline in progesterone concentration which was not accompanied by a commensurate decline in CL diameter; the CL diameter decreased from 2.2 $\pm$ 0.12 cm on day -3 to 2.0 $\pm$ 0.12 cm on day -2, while the progesterone concentration decreased from 5.4 $\pm$ 1.80 ng/ml to 0.5 $\pm$ 0.27 ng/ml. It is concluded that ultrasonic scanning coupled with hormone determination gives the most reliable evaluation of ovarian activity.

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CATTLE PRACTICE

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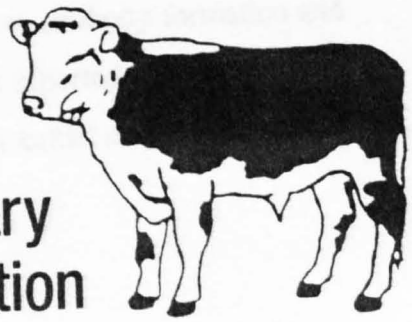

ULTRASOUND AND THE DIAGNOSIS AND TREATMENT OF OVARIAN CYSTS

KEY FACTS

- Ultrasound is useful in helping the diagnosis of ovarian cysts.
- A 7.5 MHz transducer gives a good picture of the wall and lumen of a cyst, which assists in estimation of wall thickness and confirmation of the presence of luteal tissue.
- Plasma progesterone continues to be a useful aid in diagnosis of ovarian cysts.
- Ultrasound is valuable in monitoring response of follicular cysts to treatment with GnRH, and in avoiding unnecessary repeated treatments.

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INTRODUCTION

Ovarian cysts in cattle can occur at any stage of lactation, but are common during the first 45 to 60 days after calving. They are dynamic structures which secrete different amounts of progesterone across time. A wide variation in plasma progesterone in cows clinically diagnosed with luteal cysts has been reported (Leslie and Bosu, 1983).

The feedback regulation of oestradiol on the hypothalamic-pituitary axis is altered in cystic ovarian degeneration (Dobson and Alam, 1987). Nanda et al. (1991) reported failure of LH release in response to oestradiol in 50% of cows with cystic ovarian disesase. It is not clear why only some cows with ovarian cysts respond to oestradiol. The concentration of LH in cows with ovarian cysts is higher than in of normal cows and inversely correlated to concentration of plasma progesterone (Youngquist, 1986). High progesterone concentration inhibits the positive feedback effects of oestradiol in cows (Peters, 1986).

GnRH (a decapeptide) is commonly used for the treatment of ovarian cysts. The advantages of GnRH are its low price, lack of refractoriness due to antibody formation and the fact that anaphylaxis following GnRH treatment has not been reported in cows.

Luteal tissue development following GnRH treatment in cattle may be monitored

indirectly by measurement of progesterone concentration in plasma or milk. Garverick et al. (1976), using palpation per rectum described cyst luteinization that was characterised by increased firmness and reduced cyst size in cows that responded to GnRH or human chorionic gonadotrophin (hCG) treatment. Nakao et al. (1983) found a correlation of only 30% between palpation per rectum 10 days after GnRH treatment in assessing luteinization of follicular cyst with milk progesterone analysis. Ultrasonography may allow objective monitoring of the development of luteal tissue in order to evaluate the effectiveness of treatment.

Plasma progesterone concentration is elevated 5-9 days following GnRH treatment in most cows that respond positively (Garverick et al., 1976) as a result of luteinization of the cyst or of other follicles with or without ovulation.

Ultrasonography has enabled reliable monitoring of ovarian follicular growth pattern during the oestrous cycle, but there is a paucity of information on ultrasound and progesterone monitoring of ovarian cysts and of follicles associated with cysts in cattle, hence the present study. Although endocrine changes following GnRH treatment of ovarian cysts in cattle have been described (Nanda et al. 1988), information on ultrasound changes following treatment is scarce.

The objectives of the present study were to:-

(1) examine by ultrasonography and progesterone measurement the initial occurence of ovarian cysts postpartum and the response of such cysts to exogenous oestradiol.

(2) monitor by ultrasonography and progesterone clinical cases of ovarian cysts, follicular dynamics in the presence of cysts and the response of cysts to exogenous oestradiol.

(3) monitor ovarian ultrasound changes and correlate with plasma progesterone in cows with follicular cysts treated with GnRH.

MATERIAL AND METHODS

Animals

All cows used in this study were owned by clients of the Liverpool University Farm Animal Practice in Cheshire. A total of 34 cows were used for study 1 and 2. Another 5 lactating Friesian cows with follicular cysts were used to monitor response to GnRH.

Ovarian cysts were diagnosed by palpation per rectum, ultrasonography and plasma progesterone. Palpation diagnosis was based on reproductive history (irregular oestrus, anoestrus) and palpation of a large fluid-filled structure(s) $\geq$25mm in diameter in the absence

of a corpus luteum (CL). By ultrasonography, a follicular cyst was defined as a uniformly nonechogenic ovarian structure $\geq$25mm in diameter with a wall $<$3mm thick, while a luteal cyst was a nonechogenic ovarian structure with grey patches within the antrum or along the inner cyst wall with a wall thickness $>$3mm. Plasma progesterone of $<$0.9 ng/ml and $>$0.9 ng/ml was consistent with follicular and luteal cysts respectively.

Ultrasonography

Ultrasound examination was performed using an ultrasound scanner (Aloka, BCF Technology, Livingstone, U.K.) equipped with a 7.5 MHz rectal transducer (Aloka, UST 55111, Japan). Maximum diameter of follicles $\geq$5mm, CL and inner and outer diameter of cysts were measured after freezing the image on the screen. Prints of representative images were obtained using the on-line thermal printer (Sony, Video Graphic UP-850, Japan). Ultrasonography was performed on early postpartum cows from day 7 and then weekly until day 42-49, and in cows with cysts on alternate days from day of diagnosis (day1) until day 12. Cows treated with GnRH were scanned on day of initial diagnosis (-10), day of treatment (day 0) and weekly thereafter until day 35.

Treatment

On day 11 after cyst diagnosis, all cows with luteal cysts were injected with 500 ug of Cloprostenol (Estrumate, Coopers, Pitman-Moore, U.K.) intramuscularly. On day 12 after cyst diagnosis (24 h after cloprostenol in luteal cases) all cows received 1 mg of oestradiol benzoate (Intervet, Ltd, U.K.) dissolved in 2 ml arachis oil intramuscularly. A group of 6 cyclic control cows with a functional CL were injected with cloprostenol and oestradiol benzoate 24 h later. Ten days after initial diagnosis, 500 ug of synthetic GnRH (gonadorelin, 5 ml Fertagyl; Intervet Labs Ltd, U.K.) was administered to 5 cows with follicular cysts.

Blood sampling

A blood sample was obtained after each ultrasound examination by tail venepuncture into heparinised vacutainer tubes. Further blood samples were obtained in all cows with cysts 18 h and 24 h after oestradiol.

Hormonal analysis

Plasma progesterone was measured by radioimmunoassay (Kanchev et al., 1976). The inter and intra-assay coefficient of variation were 13.6% and 8.3% respectively with a sensitivity of 0.05 ng/ml. Plasma LH was measured by a verified double antibody radioimmunoassay (Alam and Dobson, 1986). The inter and intra-assay coefficient of variation were 13.15% and 11.62% respectively. The sensitivity of the assay was 0.05 ng/ml LH per tube.

Statistical analysis

An LH surge in response to oestradiol was confirmed when LH concentration increased to over 10 ng/ml in samples collected at 18 and, or, 24 h after oestradiol. Luteal tissue volume in luteal cysts and cyst wall volume of follicular cysts was calculated by subtracting the cyst's cavity volume from total cyst volume according to the formula: $V = \pi d^3/6$, where V = volume of cyst or cyst cavity, $\pi = 3.14$, d = diameter of cyst or cyst cavity (Munday and Farrar, 1979) Correlation between ultrasound detection of CL and progesterone rise in early postpartum cows, luteal tissue volume of luteal cysts or follicular wall volume of follicular cysts and plasma prrogesterone were tested using Spearman's correlation. The mean number of all follicles $\geq$5mm in all cows with cysts over the monitoring period in the ipsilateral ovary was compared to that in the contralateral ovary using Students 't' test.

Therapeutic success was confirmed by the development of CL and/or luteinization of cysts (which was characterised by increased cyst wall thickness with granular echogenic appearance as visualised by ultrasound) and increased plasma progesterone concentration. Therapeutic failure was defined as cases in which cysts remained unchanged or new cysts developed without luteal development and the cow failed to cycle normally.

RESULTS

The relationship between follicular cyst wall volume or luteal wall volume and plasma progesterone during the monitoring period is presented in Figs 1 and 2 while Figs 3 and 4 represents mean $\pm$ S.E.M. diameters of follicles associated with follicular and luteal cysts respectively. Figs 5 and 6 shows the relationship between cyst size and progesterone concentration in GnRH-treated cows. Ovarian ultrasound findings during weekly examinations in GnRH treated cows are summarised in Table 1.

Of 18 cows monitored in the early postpartum period, 16 cows started cycling normally by day 14-28 postpartum. A CL was observed during ultrasonography in 5 cows by day 14, in 7 cows by day 21 and in 4 cows by day 28. There was complete agreement between ultrasound detection of a CL and progesterone rise in all the 16 cows that did not develop ovarian cysts. In 2 of the 16 cows however, CL and high progesterone persisted for 28-35 days. Two cows which had large follicles 20-22mm on day 35 spontaneously developed ovarian luteal cyst by day 42. The 2 cows were monitored as the clinical cases and results are presented together.

Cyst were diagnosed in all cows 80.8 $\pm$ 9.4 days postpartum. Mean cyst size on day

of diagnosis was 43.0 ± 2.8 mm for follicular and 35 ± 1.6 mm for luteal. A total of 72.2% occurred in the right ovary and 27.8% in the left ovary.

There was slight change with time in the volume of luteal tissue in luteal cysts and wall thickness volume of follicular cysts. The actual tissue volumes of both type of cysts were in similar ranges (10.46 ± 1.34 x to 14.09 ± 1.41 ± x 10^3 mm^3 for luteal and 10.38 ± 3.44 to 14.24 ± 4.95 x 10^3 mm^3 for follicular cysts. The luteal tissue volume decreased slightly 24 h after cloprostenol but was still visible on ultrasonography (though less homogenous). A correlation of 0.54 was obtained between luteal tissue volume and plasma progesterone. There was no correlation between cyst wall volume of follicular cysts and plasma progesterone.

Progesterone concentration in all cows with follicular cysts remained low (<0.9 ng/ml). In cows with luteal cysts, progesterone concentration ranged between 2.78 ± 0.83 and 4.86 ± 0.92 ng/ml. However, 24 h after prostaglandin progesterone fell from 2.78 ± 0.83 to 0.63 ± 0.15 ng/ml.

There were significantly ($P<0.05$) more follicles in the contralateral ovary than in the ipsilateral ovary (irrespective of cyst type). No clear wave pattern of follicular growth was discernible in the presence of cysts in this study.

Five of 6 (83.3%) control cows had an LH surge at 18 and, or, 24 h after exogenous oestradiol administration. Plasma progesterone in all 6 cows was low (<0.9 ng/ml) at the time of oestradiol treatment. One of 7 (14.3%) follicular cysts and 3 of 11 (27.3%) luteal cysts responded with surge levels of LH (>10 ng/ml) to exogenous oestradiol treatment. One follicular cyst and 1 luteal cyst had surge levels at the time of oestradiol treatment.

Prior to GnRH treatment 3 cows showed recurrent oestrous behaviour and 2 cases showed no oestrus. All five responded positively to GnRH treatment. By day 7 after treatment, luteinization of cysts was noted during ultrasonography in 2 cows while GnRH-induced CL were observed in the remaining 3 cows.

Changes noted during weekly ultrasonography after treatment included clouding of the uniformly nonechogenic antrum of cysts, luteinization of cyst walls, reduction in cyst size or resolution of cyst, and, or, development of 1-4 CL lutea in the ovary bearing the cyst and/or in the contra-lateral ovary. All 5 cows were in oestrus 18.80 ± 1.74 days after treatment.

Mean (± S.E.M.) plasma progesterone concentration on day -10 and day 0 were 0.2 ± 0.13 ng/ml and 0.72 ± 0.27 ng/ml respectively. Progesterone concentration was elevated

(5.8 ± 0.67 ng/ml) in all cows by day 7.

DISCUSSION

The early postpartum study showed that 16 of 18 cows failed to develop ovarian cysts. Of these, (31%) had a rise in progesterone by 14 days postpartum, 7 (44%) by day 21, and 4 (25%) by day 28. There was complete agreement between ultrasound detection of CL and a rise in plasma progesterone. Lamming and Bulman (1976) reported significant rises in milk progesterone in 50% of cows before day 20 postpartum and ovarian activity had commenced in 93% by day 40 postpartum. Persistent CL accompanied by high progesterone was noted in 2 cows in this study. Lamming and Bulman (1976) similarly observed persistently high milk progesterone for at least 30 days in 2% of cows studied.

The number of cows that developed ovarian cysts during the postpartum monitoring (11%) in the present study was lower than figures reported previously, e.g. 24% by Carroll et al., (1989).

A correlation of 0.54 was obtained between luteal tissue volume and plasma progesterone. There was however no correlation between follicular cyst wall volume and plasma progesterone. This was not surprising because follicular cysts tended to be larger and the cyst wall volume could be substantial, but, as a result of lack of luteinization progesterone remained low (<0.9 ng/ml). Plasma progesterone in luteal cases fell from 2.78 ± 0.83 ng/ml to 0.63 ± 0.15 ng/ml within 24 h of cloprostenol injection. Luteal tissue was however, visible during ultrasonography (though slightly less homogenous). This re-emphasises the point that morphological presence is not always equated with physiological function. Plasma progesterone associated with follicular and luteal cysts in this study were consistent with previous reports (Leslie and Bosu, 1983).

The observation in this study for the first time of significantly ($P<0.05$) more follicles in the contralateral ovary than in the ovary ipsilateral to the cyst (irrespective of cyst type) suggests that the presence of a cyst may be detrimental to the development and maturation of other follicles on that ovary. More detailed study is required in this direction.

The hypothalamic-pituitary feedback of control and cystic cows was evaluated in this study by measuring increase in plasma LH concentration after exogenous oestradiol. Overall, 4/18 (22%) LH response was obtained. This was higher than the figures reported by Dobson and Alam (1987) but lower than the 50% response by Nanda et al. (1991). The difference may be attributed to difference in number of and types of cases. A surge of LH was

characterised in 5/6 (83%) control cows. The failure of 1 control cow to respond could not be explained: progesterone at the time of oestradiol was <0.9 ng/ml.

Nanda et al. (1991) suggested that progesterone pretreatment of ovarian cysts appeared to heal the prevailing endocrine lesion and improve LH response to oestradiol, and that failure of 53% of luteal cysts in their study to respond to oestradiol may have been due to short term (less than 7 days) exposure to progesterone (Nanda et al., 1988). In the present study, 73% of luteal cysts failed to respond to oestradiol even though functional luteal tissue was present in all for at least 11 days before prostaglandin. It may be that a factor in the persistence and perhaps initiation of the cystic condition is refractoriness to the positive feedback effect of oestradiol on gonadotrophin release (Refsal et al., 1988).

The visualisation by ultrasonography of cyst luteinization and, or, the presence of CL in all cows by day 7 after GnRH shows that in responding cases, evidence for therapeutic success could be confirmed quickly. All five cases in this study responded positively by either cyst luteinization and/or CL formation. Failure to detect luteinization of follicular cysts accurately after GnRH by palpation per rectum has led to unnecessary treatment in many cows. Ultrasonography could prevent this problem. It has been reported that GnRH has about 80% success rate in treating ovarian cysts in cattle (Kesler and Garverick, 1982).

Exogenous GnRH acts on the pituitary gland to cause release of endogenous LH (Garverick et al., 1976). Response of cows with ovarian cysts appears to vary with the dose of GnRH administered (Youngquist, 1986). If sufficient GnRH is given and mature follicles are present, ovulation is induced within 24 - 30h.

The observation of an increase in progesterone by day 7 after treatment is consistent with previous studies (e.g. Garverick et al., 1976). Progesterone concentration remained low (<0.9 ng/ml) in all cows from day -10 to day 0. Cantley et al. (1975) found higher mean pre-treatment concentration of progesterone in cows that responded to GnRH than in those that did not respond.

In conclusion, spontaneous luteinization of follicular cysts was not observed in this study. Response of cows with cysts to exogenous oestradiol was variable. The presence of a cyst seemed to be detrimental to the development and maturation of follicles in same ovary. It is evident that ultrasonography and progesterone measurement are useful tools for evaluating the response of follicular cysts to GnRH in cows.

Fig 1: Dynamics of ovarian follicular cysts (n=7)

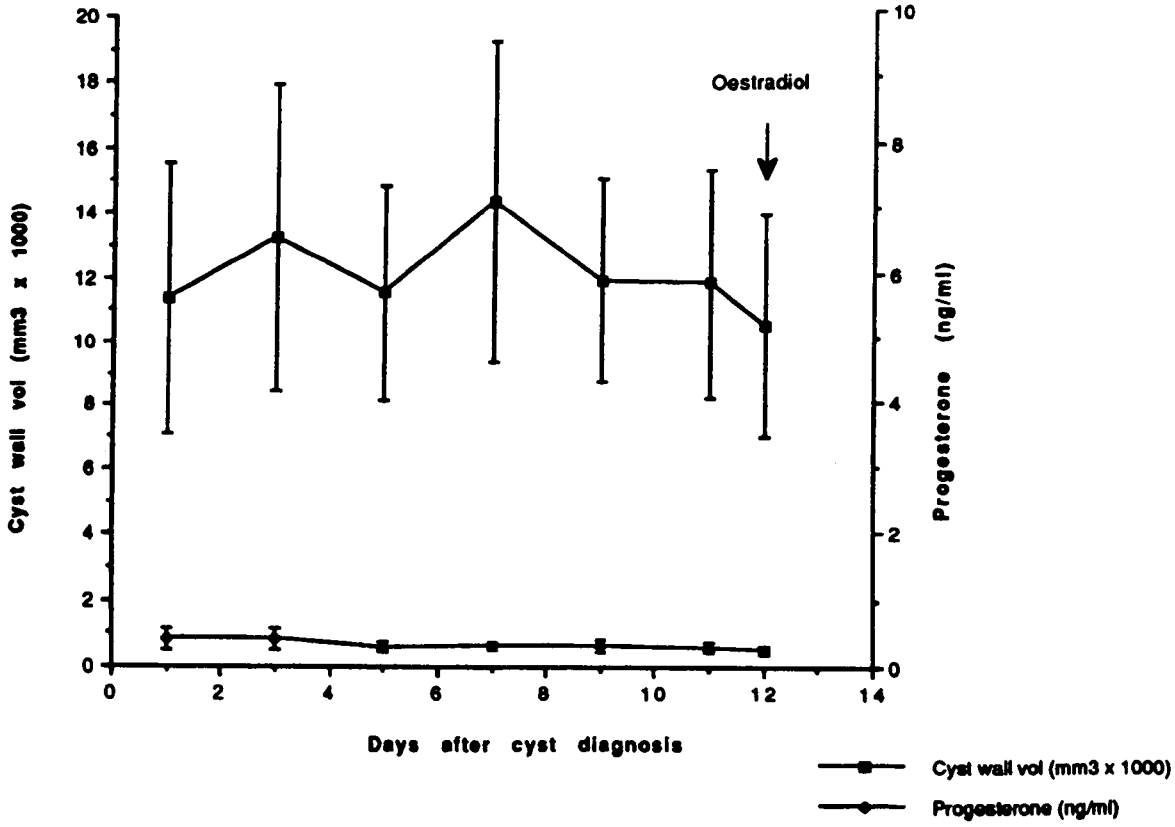

Fig 2: Dynamics of luteal ovarian cysts in cows (n=11)

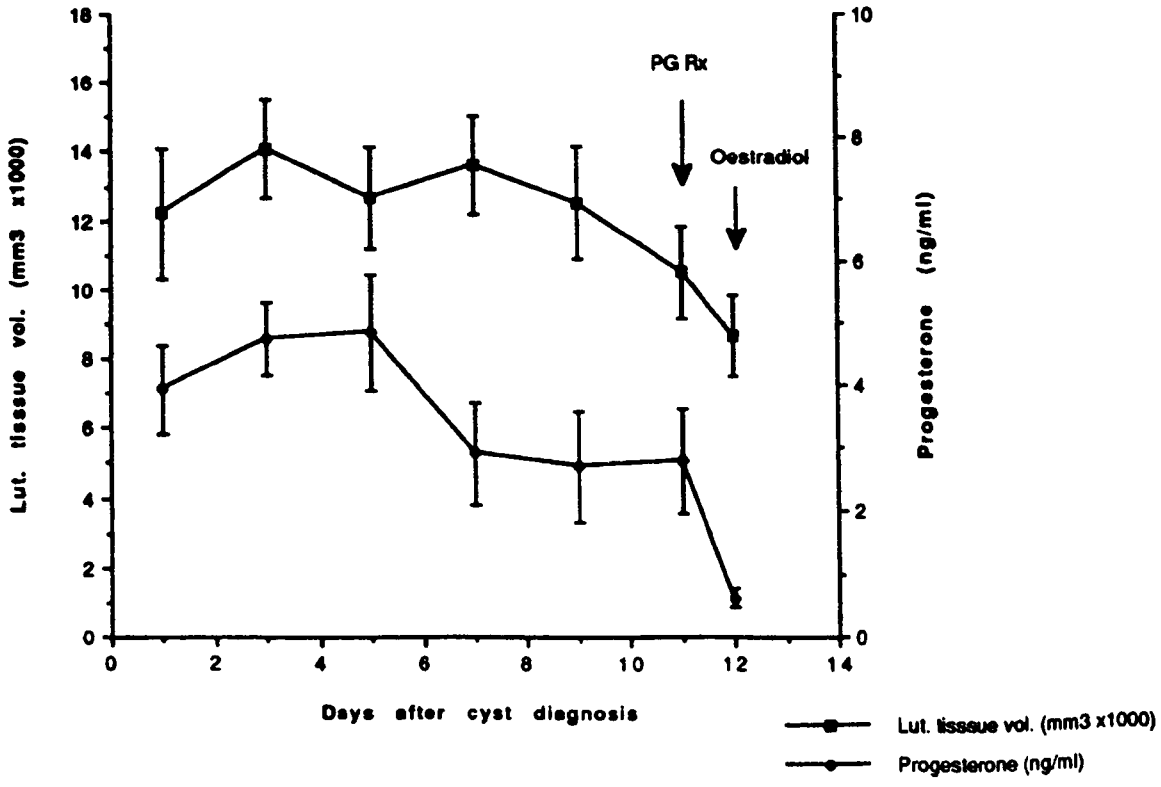

Fig 3: Mean (± SEM) sizes of follicles and follicular cysts (n=7)

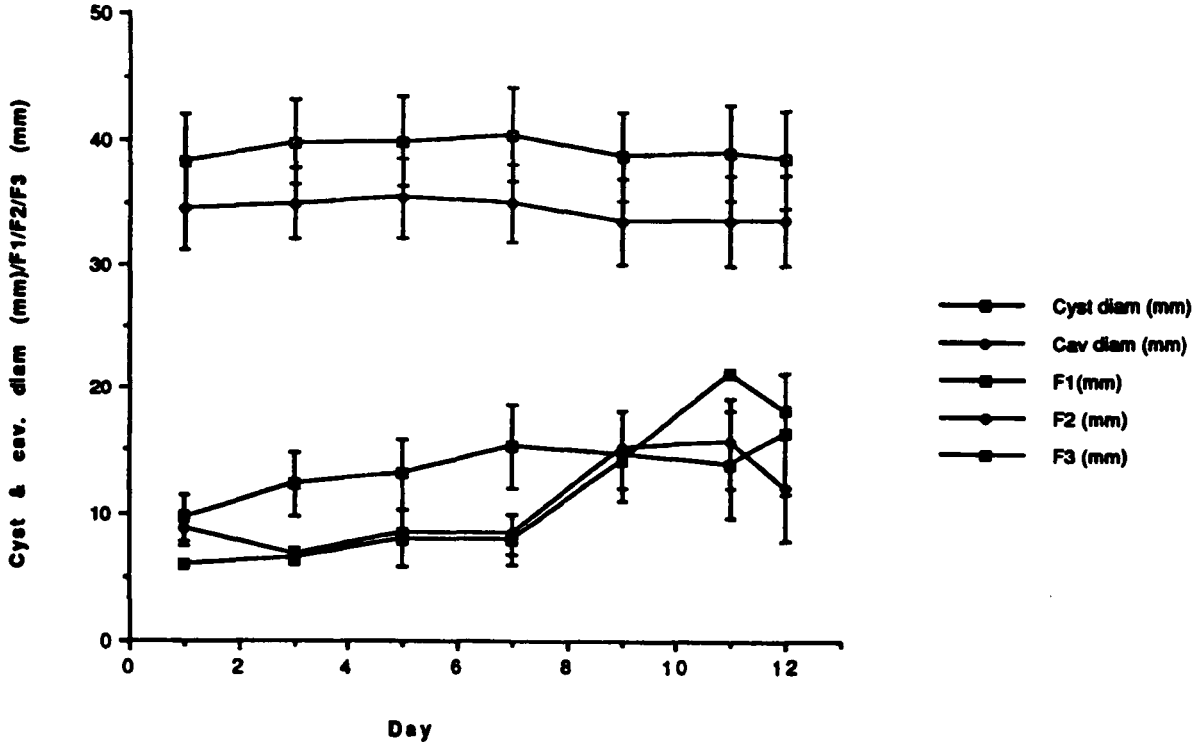

Fig 4: Mean (±SEM) diameter of follicles with luteal cysts (n=11)

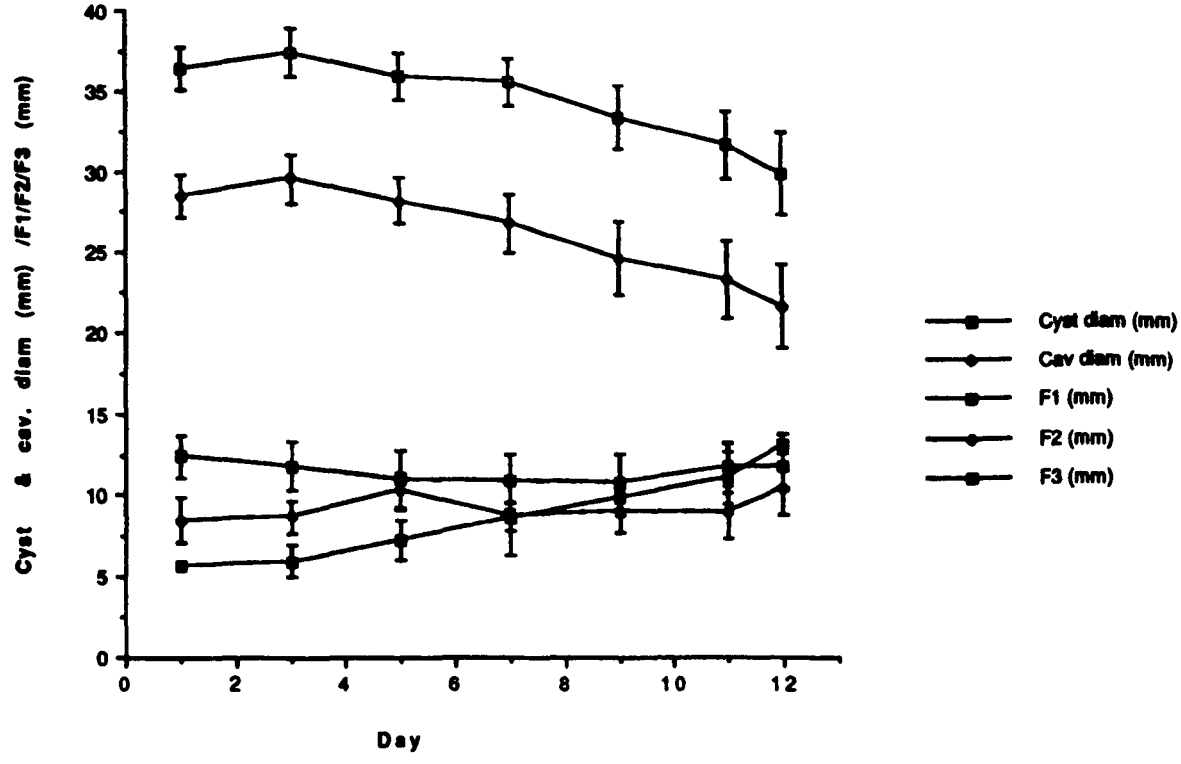

Fig 5: Dynamics of follicular cysts that initially responded by cyst wall luteinization (n=2)

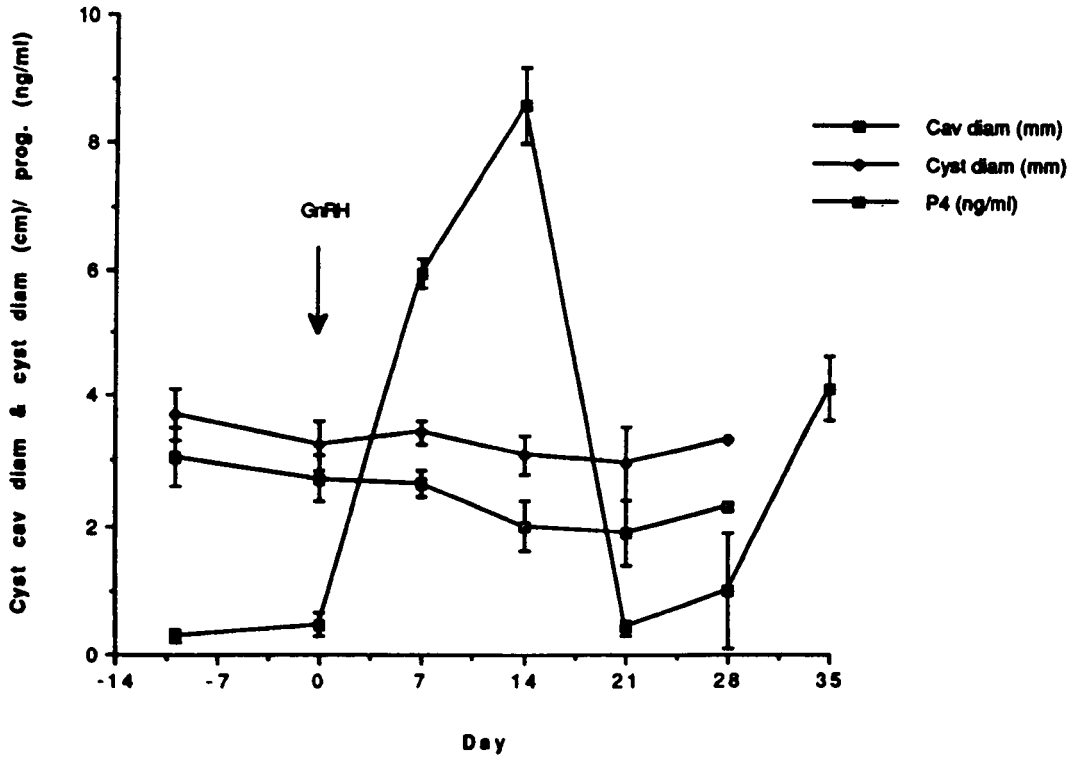

Fig 6: Dynamics of follicular cysts that initially responded by CL development (n=3)

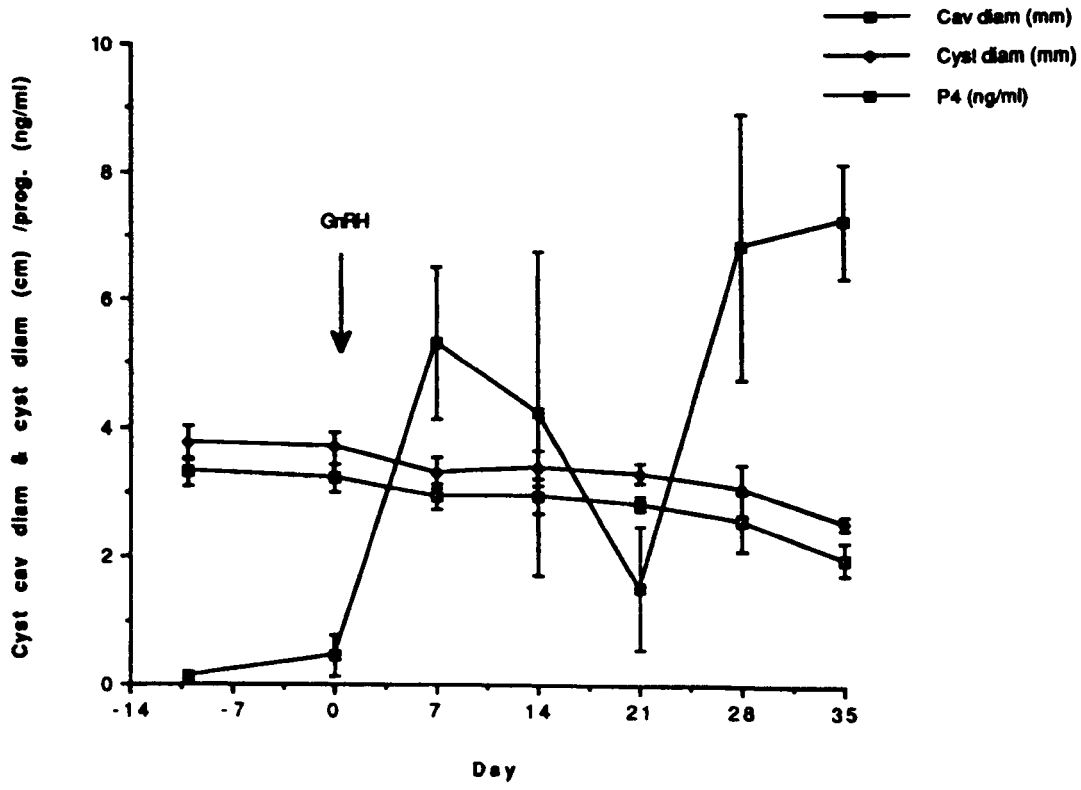

Table I. Ultrasound findings on both ovaries before and after treatment of follicular cysts in cows (n=5) treated with GnRH

Cow	Day -10	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35
799	FC + 2F	FC + 3F	FC + 4CL	FC + rCL	FC + CL	FC + CL	rFC + CL
72	FC + F	FC + 3F	Lut.FC +Lut.F	Lut.FC + Lut. F + CL	Lut.FC + Lut. F	Lut.FC + CL + F	F + CL
145	2FC + F	2FC + F	2FC + F + CL	2FC + CL	2FC + F + rCL	FC + 4F + CL	3F + CL
603	2FC + F	2FC + 2F	2FC + CL	2FC + CL	2FC + F	FC + F + CL	rFC + F + CL
457	FC + 2F	3FC	3Lut.FC + F	3Lut.FC	rLut.FC +F	CL	F + CL

FC = Follicular cyst

F = Follicle $\geq$ 10mm

rFC = Regressing follicular structure <25mm

Lut.FC = Luteinized follicular cyst (cyst wall >3mm thick)

Lut.F = Luteinized follicle (large follicle with a thick wall >3mm)

rLut.FC = regressing luteinized follicular cyst

CL = Corpus luteum

rCL = regressing CL

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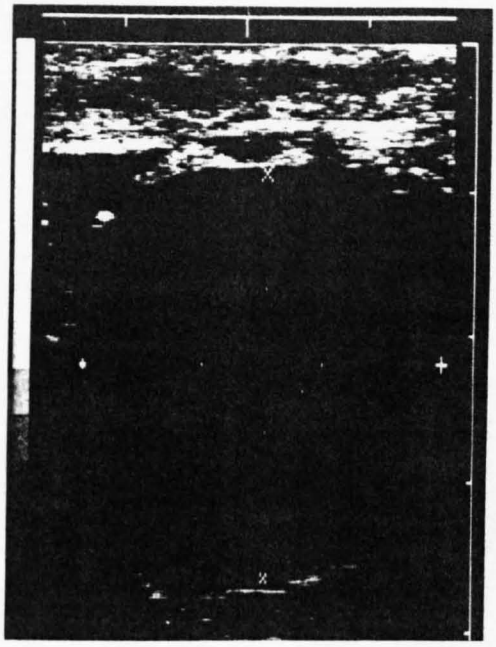

Fig 7: Ultrasonogram of a follicular cyst

Cyst cavity diameter = 29mm

Cyst diameter = 31mm

Progesterone concentration = 0.17 ng/ml

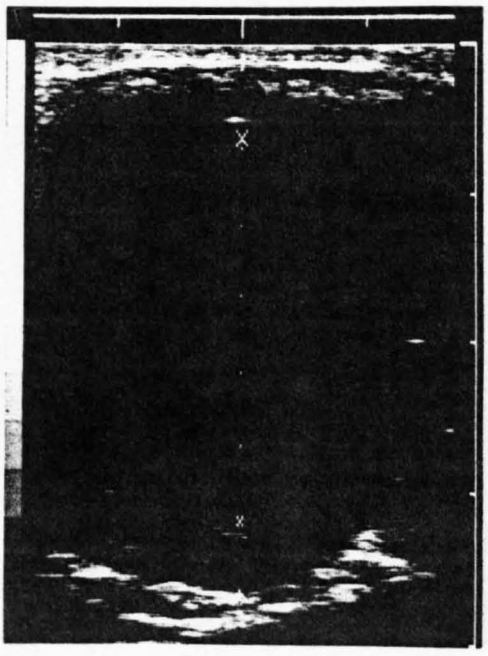

Fig 8: Ultrasonogram of luteal cyst

Cyst cavity diameter = 26mm

Cyst diameter = 36mm

Progesterone concentration = 2.72 ng/ml

BJR

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Volume 66 Number 787 July 1993

Ultrasound Special Issue

Including papers from the Twenty-fourth Annual Meeting of the British Medical Ultrasound Society, held at the Blackpool Wintergardens, Blackpool, December 9–11, 1992

Guest Editors:
H C Irving and W N McDicken

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centres with differing requirements. Once the QA method itself had been fully developed a more flexible computer package was required to facilitate dissemination. The requirements of this package were that it should be transferable to IBM-compatible PCs, and it should be user-configurable in terms of the number of sonographers, local measurement methods, quality criteria and report formats. The latest version of the software is described. It is in a modular form with configuration, data input, analysis and reporting routines linked by an external menu system. The number and nature of quality criteria are user selectable, defaulting to those developed in our centres; the system may very easily be configured to include any future nationally agreed standards.

Fetal penile length

P Johnson and D Maxwell
Fetal Medicine Unit, Guy's Hospital, London SE1 9RT, UK

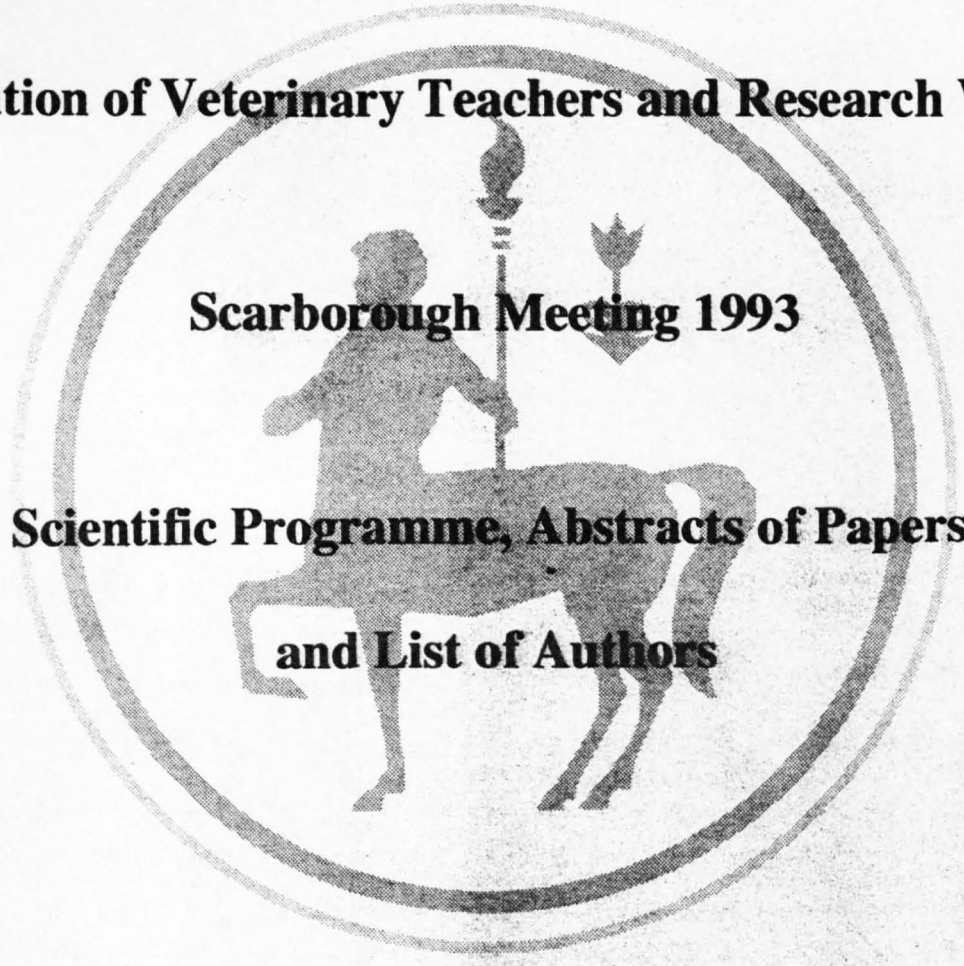
The differential diagnosis of fetal bladder outflow obstruction includes urethral agenesis and posterior urethral valves. These conditions have markedly different prognoses but differentiating between them using prenatal ultrasound is difficult. However, cases of urethral agenesis frequently have major distortion of penile anatomy, and therefore measurement of penile length may help in this diagnostic situation. The penile length of 91 male fetuses scanned in the Fetal Medicine Unit was recorded in order to establish a reference range. In each case, three measurements within 1 mm were required. Gestational age was taken from menstrual dates if these were confirmed by ultrasound measurements of BPD, HC, AC and FL. The fetuses examined were between 16 and 40 weeks gestation. Penile length increases significantly with age, with a mean of 6.1 mm at 16 weeks and 28.27 mm at 38 weeks (log (length) = $0.3 + 0.303 \times$ gestation; $p = < 0.001$). In two cases of bladder outflow obstruction penile length was measured. Case A had a penis of 8 mm at 19 weeks (on 50th centile), and postnatal examination confirmed posterior urethral valves. Case B had a penis of 3 mm at 18 weeks gestation (below 5th centile), and postmortem examination confirmed urethral agenesis with severe epispadias. There is clinical value in the measurement of penile length.

Comparative diagnosis of follicular and luteal ovarian cysts in cattle by palpation per rectum and ultrasonography

A Y Ribadu, W R Ward, L Oliveira and H Dobson
Department of Veterinary Clinical Science, University of Liverpool, Leahurst, Neston, South Wirral L64 7TE, UK

The purpose of this study was to compare (using progesterone values) the accuracy of palpation per rectum and ultrasonography for diagnosis of follicular and luteal ovarian cysts in cattle. 29 cows were diagnosed by palpation per rectum as having either follicular or luteal cysts by four experienced veterinarians on herd health visits. During ultrasonography, an ovarian cyst was defined as a nonechogenic structure > 25 mm in diameter irrespective of the presence of other ovarian structures. Cysts were further classified into follicular (uniformly nonechogenic antrum with a thin wall < 3 mm) or luteal (nonechogenic antrum with grey patches within the antrum or along the inner cyst wall with a thick wall > 3 mm). Ultrasound diagnosis was independent of palpation per rectum diagnosis. Correct diagnosis of follicular cyst was based on ultrasound finding of a nonechogenic structure > 25 mm with plasma progesterone concentration < 0.9 ng ml^{-1}. Luteal cysts were confirmed based on ultrasound finding of nonechogenic ovarian structures > 25 mm with plasma progesterone concentration > 0.9 ng ml^{-1}. Overall, palpation per rectum correctly diagnosed ovarian follicular and luteal cysts in 15 out of 29 cows (52%). Ultrasonography correctly determined the presence of ovarian cysts in 15 cows, dominant follicles (diameter 12–14 mm) in three cows and corpora lutea (with or without cavities) in the remaining 11 cows. Mean plasma ($\pm$SEM) progesterone concentration was 0.1 ± 0.03 ng ml^{-1} for cows with follicular cysts ($n = 5$), 3.6 ± 0.49 ng ml^{-1} for cows with luteal cysts ($n = 10$) and for the three cows with dominant follicles and 11 cows with corpora lutea were 0.46 ± 0.05 ng ml^{-1} and 6.15 ± 0.50 ng ml^{-1} respectively. Corpora lutea and cystic CL accounted for 79% of misdiagnosis by palpation per rectum while dominant follicles constituted the remaining 21%. Ultrasonographic classification of cyst type was consistent with progesterone classification in 93%. In conclusion, ultrasonography is a very accurate method for diagnosis of ovarian cysts in cattle compared with palpation per rectum. It offers a useful and reliable diagnostic tool.

Association of Veterinary Teachers and Research Workers



Scarborough Meeting 1993

Scientific Programme, Abstracts of Papers and List of Authors

A Division of the British Veterinary Association

Room A.
Morning Session

9.00 9.20
1A01 1A02

Ultrasonography Of Placental Lesions In Ewes With *C.psittaci* Infection.

A. Ortega–Pacheco*, H. Philips, M. J. Clarkson and R. D. Murray,
Department of Veterinary Clinical Science and Animal Husbandry, Leahurst, Neston, South Wirral, L64 7TE.

Ultrasonography has been used extensively in veterinary reproductive science. Diagnosis of disorders (such as pyometra and ovarian cysts) of the reproductive tract has been carried out successfully in cattle using this method (Fissore *et al*, 1986). No studies have identified placental lesions associated with infectious agents responsible for abortion or birth of premature lambs.
Chlamydia psittaci produces a disease of pregnant sheep characterised by abortions without well defined clinical signs. Pathological changes are not detected until 90 days of gestation; placentitis is a consistent feature and necrosis may affect the entire cotyledon or its periphery.
Five Welsh crossbreed sheep were orally inoculated during the last trimester of pregnancy with Chlamydia abortion isolates. Trans–abdominal real–time ultrasound examination, using a 5MHz transducer (Aloka), was started a week after inoculation and continued twice weekly thereafter. Results showed that placental lesions were present, associated with EAE infection, and that these lesions can be monitored by this technique before either abortion or premature birth of live lambs occurs.

Retrospective Investigation Of Subfertility In A Dairy Herd Associated With *L.hardjo* Infection.

G. S. Dhaliwal*, R. D. Murray, H. Dobson and A.Ortega–Pacheco.
Department of Veterinary ClinicaL Science and Animal Husbandry, Leahurst, Neston, South Wirral, L64 7TE.

Fertility records of a herd experiencing subfertility during the last eight years were investigated. Results of diagnostic investigations revealed chronic *L.hardjo* infection. Agalactia and abortions occurred prior to a vaccination control programme; abortions continued even after the vaccination regime was in place. Two years after the last vaccination there was a serological prevalence within the whole herd of more than 80%. Reproductive performance was analysed throughout the 8–year period, measured by (i) calving to conception interval, (ii) services per conception and (iii) first service conception rate. These parameters were below acceptable limits and did not alter significantly following vaccination. However, decline in reproductive performance, as measured by herd fertility status (HFS), was evident during the years when clinical disease associated with *L. hardjo* infection was present. Inter–service intervals were extended with more than 50% falling in the category beyond 48 days. The epidemiological pattern of disease will be discussed, together with the methods of analysis used.

9.40 10.00
1A03 1A04

Comparative Detection And Classification Of Corpora Lutea By Palpation Per Rectum And Ultrasonography In Cows.

A.Y. Ribadu*, H. Dobson and W.R. Ward.
Department of Veterinary Clinical Science & Animal Husbandry, University of Liverpool, Leahurst, Neston, L64 7TE.

The accuracy of palpation per rectum and ultrasonography for determining the presence and stage of corpora lutea (CL) was investigated in 34 cows using the results of dissection of ovaries postslaughter and plasma progesterone (P4) concentration as the "gold standard". Corpora lutea were classified as either young (days 1–4), mature/mid–cyclic (days 5–16) or old (days 17–21) according to morphological criteria. Transrectal ultrasound examination was carried out using a realtime ultra–sound scanner equipped with a 7.5MHz linear array rectal transducer. Plasma P4 concentration was determined by radio–immunoassay. There was complete agreement between dissection and P4 classification of CL. Neither palpation per rectum nor ultrasonography detected any young CL which was also confirmed by ovarian dissection.The sensitivity, specificity and positive predictive value of palpation per rectum for identifying mature CL was 85%, 95.74% and 89.47% respectively. Ultrasonography on the other hand had a sensitivity of 95%, specificity of 100% and positive predictive value of 100%. Plasma P4 concentration (mean ± S.E.M) in cows with mature CL was 6.13 ± 0.67ng/ml while in cows with old CL (n=3) and those without CL (n=11) were 0.22 ± 0.11ng/ml and 0.10 ± 0.02ng/ml respectively. The weights of mature CL ranged between 2.17 and 8.86g. Ultrasonography detected double CL (n=3) and CL with cavities (n=2) which were subsequently confirmed by dissection. Of 3 old CL (associated with low P4), none was detected by ultrasonography while one was wrongly classified by palpation per rectum as a mature CL. There was no significant difference ($P>0.05$) between palpation per rectum and ultrasonography for identifying mature CL in bovine ovaries.

Ultrasound Monitoring of Ovarian Changes in Cattle After Injection of Buserelin 11–13 Days Post–Insemination.

L. Robertson, M. Salaheddine and M.J.A. Harvey.
Department of Surgery and Reproduction, University of Glasgow Veterinary School, Glasgow G61 1QH.

Recent work has suggested that injection of a GnRH analogue 11–13 days post–insemination results in a 10% increase in conception rate of cows. This is associated with the maintenance of an elevated plasma progesterone concentration over the critical period of maternal recognition of pregnancy. While the mechanism underlying this response has not been elucidated it has been suggested that the GnRH analogue may boost function of the existing corpus luteum, cause luteinisation of a follicle or cause a further ovulation and formation of a new corpus luteum. This study used transrectal ultrasound to monitor ovarian changes subsequent to injection of 10µg Buserelin (Receptal; Hoescht) 11–13 days post–insemination. Of 16 cows examined, 5 cows formed a new luteal structure after injection. In the one cow which was sacrificed this structure was a new corpus luteum. The other 11 showed no visible ovarian changes. These results indicate that injection of the GnRH analogue can cause formation of a new luteal structure. However, since this did not occur in every case this is not the only mechanism of drug action.

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145

Ultrasonographic study of ovarian follicular dynamics in the ewe

JP Ravindra, NC Rawlings, ACO Evans, GP Adams. WCVM, University of Saskatchewan, Saskatoon, Sask. CANADA S7N 0W0.

Transrectal ultrasonographic examination of 8 white face cross bred ewes was done daily throughout an oestrous cycle to follow the ovarian follicular events. A rigid, externally held 7.5 MHz human prostate transducer was used, with the ewe in a standing position. Daily blood samples were collected for hormone analysis. Follicles with a diameter of 2 mm or more were detected on both ovaries and recorded for their position and size. The inter-ovulatory interval was 17.2 ± 0.4(mean±sem) days. Except for Days 2.7 ± 0.5 and 11.1 ± 0.3, when all the ewes showed emergence of follicles, there appeared to be a random emergence of follicles through the cycle. The ovulating follicle was retrospectively traced to emergence on Day 11.1 ± 0.3 and grew at a rate of 1.1 ± 0.1 mm/day to a maximum diameter of 6.9 ± 0.1 mm before ovulating. Follicles that did not ovulate attained a maximum diameter of 4.2 ± 0.4 mm, except for those emerging on day 11.1 ± 0.3, which reached a diameter of 5.0 ± 0.3 mm. The regression rate of non-ovulating follicles was 1.3 ± 0.1 mm/day. Follicles numbers increased over the oestrous cycle to 7.2 ± 0.7 on day 11 and a sharp decline could be seen starting on Day 15, to reach a low of 3.5 ± 0.3 on day 17. Hormone analysis did not indicate any major increases in FSH during the oestrous cycle, except at oestrus.

We conclude that ovarian follicles grow and regress throughout the oestrous cycle of the ewe, emerging randomly except for Days 2.7 ± 0.5 and 11.1 ± 0.3 when there was consistency between ewes in emergence of follicles. While the decline in number of follicles that occurred late in the cycle nearing ovulation indicated dominance by the ovulating follicle, no clear wave pattern of growth and regression was seen and no evidence of follicular dominance was seen at other stages of the cycle in contrast to cattle.

146

Ultrasound and hormone study of cystic ovaries in postpartum cows

A.Y. RIBADU, W.R. WARD and H. DOBSON, Department of Veterinary Clinical Science & Animal Husbandry, University of Liverpool, Leahurst, Neston, L64 7TE, U.K.

Initially, 18 cows were monitored by weekly ovarian ultrasonography and plasma progesterone (P_4) determination from day 7 to 49 postpartum. A cyst was defined as a nonechogenic ovarian structure >25mm diameter that persisted for 10 days or more. Forty two days postpartum, 2 cows spontaneously developed ovarian luteal cysts (nonechogenic antrum with a wall >3mm thick and plasma P_4 >0.9 ng/ml).

In a second study, 16 clinically-presented cases (>42 days postpartum) were monitored on alternate days for 14 days. There was considerable variation in the amount of luteal tissue and plasma P_4 in the 9 cases of luteal cysts. Plasma P_4 in the 7 follicular cysts remained consistently low (<0.9 ng/ml) and the thickness of the wall did not change; in cyclic cows normal follicles (13-20mm) were present for <6 days.

In another study, 5 cows with follicular cysts were treated with GnRH (0.5mg) 14 days after diagnosis and subsequently monitored for 35 days. Changes noted on weekly ultrasound included clouding of the uniformly nonechogenic antrum, luteinization of the cyst, reduction in cyst size and/or development of 2-4 CL in the ipsi- or contra-lateral ovary. Plasma P_4 increased in all cows by day 7 after treatment.

In conclusion, abnormal follicular development in bovine ovaries was demonstrated by ultrasonography and plasma P_4.