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ACCURACY OF RECTAL PALPATION IN COMBINATION WITH ULTRASONOGRAPHY TO DETERMINE CYCLE STAGE IN DAIRY COWS

by

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Research as part of Master's dissertation

FOREWORD

Making this thesis would not have been possible without the help of others. Therefore I would like to say a word of thanks to these people.

First of all, I would like to thank my promoter Professor Dr. Geert Opsomer for the many hours he was able to work with me, both inside and outside the slaughterhouse. Although he is a very busy man, he always made time to help me and answer my questions.

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ABSTRACT

In the slaughter house, dairy cows with unknown reproductive history have been examined with the anamnesis of ‘not seen in estrus’. The purpose of the study was to 1) set the diagnosis and determine the accuracy of rectal palpation and transrectal ultrasonography compared to inspection of the ovaries after slaughter and 2) determine the cycle stage in cycling animals.

The estrous cycle of the cow was divided into three stages based on the findings of other authors. During the first part of the study 23 cows were examined, first by rectal palpation and then by transrectal ultrasonography. The combination of these two exams had to determine the cycle stage of each cow. After slaughter the ovaries were inspected and again the cycle stage was determined. The two results were compared with each other. As the division into the different stages was not always clear, another 26 cows were examined. This time the definitions of the three stages were not followed strictly and rectal palpation was sometimes carried out a second time (after ultrasonography) in case of doubt.

Ultimately no accuracy was calculated because of the limited number of cycling animals in this study. It can be concluded however, that mid-cycle CLs can be easily identified by rectal palpation and ultrasonography. Several problems occurred when dealing with developing and regressing CLs. More studies are needed in order to develop clearer ‘rules’ about how to accurately determine the cycle stage in cows.

SAMENVATTING

In het slachthuis zijn melkkoeien, met onbekende voortplantingsgegeven en als anamnese ‘niet tochtig gezien’, onderzocht. Het doel van de studie was om: 1) gebruik makend van rectaal onderzoek en echografie de diagnose te stellen en de accuraatheid ervan te bepalen met inspectie van de ovaria na het slachten als goudstandaard en om 2) het cyclus stadium van cyclerende koeien te bepalen.

De cyclus van het rund werd verdeeld in drie stadia gebaseerd op de bevindingen van andere auteurs. Gedurende het eerste deel van de studie zijn 23 koeien onderzocht, eerst via rectale palpatie, vervolgens via transrectale echografie. Doel van het onderzoek bestond er in om via de combinatie van deze twee onderzoeken het cyclusstadium van de koe exact in te schatten. Na het slachten werden de ovaria geïnspecteerd en werd opnieuw het cyclusstadium bepaald. De twee resultaten werden met elkaar vergeleken. Omdat er soms wat problemen waren bij het indelen in de stadia, werden nog 26 koeien onderzocht. Dit keer werden de definities van de drie stadia niet meer zo strict gevolgd en soms werd er nog een tweede keer rectaal opgevoeld (na de echografie) als er twijfel was.

Uiteindelijk werd er geen accuraatheid berekend vanwege het beperkt aantal cyclerende dieren in deze studie. Er kan echter wel geconcludeerd worden dat CLs in het midden van de cyclus makkelijk geïdentificeerd kunnen worden door middel van rectale palpatie en echografie. Er waren wel een aantal problemen met het correct identificeren van zich ontwikkelende en regresserende CLs. Als conclusie kan dan ook gesteld worden dat er meer onderzoek nodig is om duidelijkere ‘regels’ op te stellen over het bepalen van het cyclusstadium bij cyclerende koeien.

1. INTRODUCTION

One of the main challenges in modern dairy farming is to attain an efficient rate of reproduction. To achieve this it can be of great importance for veterinary practitioners to identify and differentiate between the different stages of the estrous cycle. The correct diagnosis of the cyclic stage is needed not only to determine when a cow in subestrus will have her next ovulation, but also to know if an injection with prostaglandins (PG) will have any effect on the present corpus luteum (CL).

Rectal palpation is still the most commonly used diagnostic technique to examine a cow's genital tract, but it is well documented its findings are far from 100% correct. Pieterse et al. (1990) determined an accuracy of 93% for palpation of a CL, although he found this number depends on the cycle stage the cow is in: for mid-cycle CLs the results have been found to be better than early or late-cycle CLs. As expected, the experience of the examiner also plays a major role.

The use of ultrasound has increased dramatically during the last decade. As with rectal palpation, ultrasound results can be influenced by the cycle stage of the cow. Many studies regarding the accuracy of rectal palpation and ultrasonography have been performed. However there is very little research available on the level of accuracy achievable through a combination of these two techniques. The objective of this thesis is to estimate the accuracy of rectal palpation in combination with transrectal ultrasonography. Post mortal dissection of the genital tract has been used as the golden standard.

2. LITERATURE REVIEW

2.1 Anatomy of the genital tract

2.1.1 General

The female genital tract consists of different organs (see Figure 1) (Simoens, 2005; Budras and Habel, 2003). The vulva (or lips) forms the external opening and is the entry way into the vestibule of the vagina, which is located on the bottom of the pelvic canal. In the floor of the vestibule, 7-11 cm cranial from the ventral commissure of the labia, the urethra appears at what is called the external urethral orifice. Just posterior to this is a blind pouch, the sub-urethral diverticulum. Also in the vestibule lie the greater vestibular glands, or Bartholin's glands. Cranially the vestibule joins the vagina. These two organs are sometimes divided by the hymen, a fold of mucous membrane. The vagina is about 30 cm long and functions as birth canal and as copulatory organ. Its fornix arches over the portio vaginalis cervicis, which is formed by the last circular fold (see further on), dorsally. The vaginal epithelium near the cervix produces mucus, particularly around the time of estrus (Ball and Peters, 2004a). The uterus is of the bipartite type and consists of a cervix, a short body, and two uterine horns. The cervix, which is 8-10 cm long in the mature cow and has a diameter of 2-7 cm, connects the vagina with the uterine body and has a very thick fibrous wall. Its mucosa contains longitudinal folds and four circular folds that close its lumen, thereby separating the sterile uterus from the non-sterile vagina. The horns of the uterus are 30-40 cm long and are, cranially from the body of the uterus, connected by the dorsal and ventral intercornual ligaments. Caudally they are fused into a 10-15 cm long double cylinder. The uterine horns have the general shape of ram horns: they bend in ventrolateral direction, go further in caudal direction and the top of the horns point up again. The uterine body and each of the uterine horns contain four rows of 10-15 caruncles of various sizes. During pregnancy these will grow and become of utmost importance for feto-maternal contact. The endometrial glands grow and secrete as the level of progesterone produced by the developing CL rises, returning to their basal size once the first signs of luteal regression are noted. The oviducts or fallopian tubes are 15-30 cm long. They catch and transport the ovulated eggs from the ovaries to the uterus, and are also the place where fertilization takes place. The cow's ovaries are relatively small (4 x 2.5 x 1.5 cm without any functional structures) and lie near the lateroventral part of the pelvic inlet. Several factors e.g. the presence of a CL or cyst can increase the overall size of the ovary.

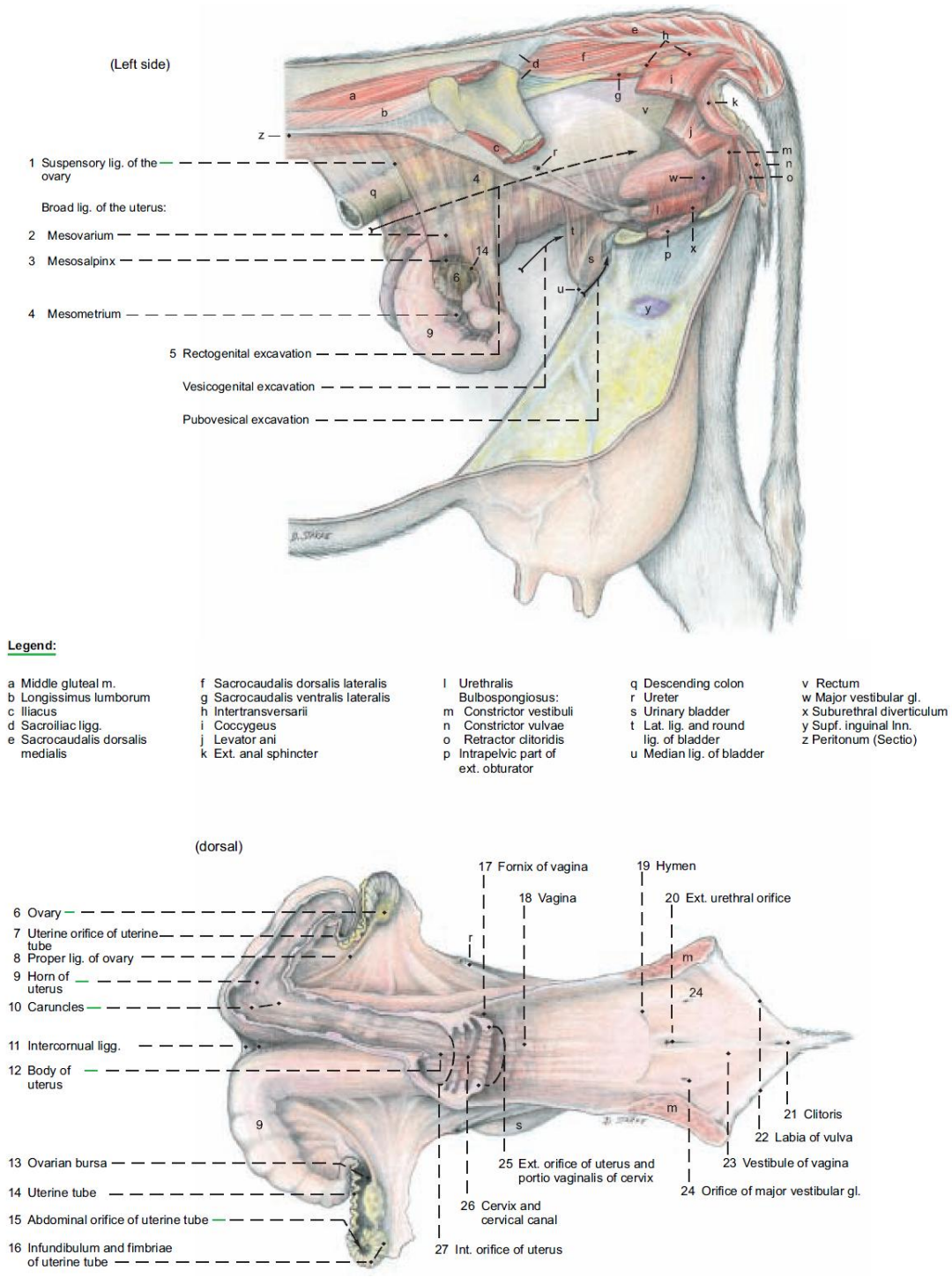

Figure 1. Anatomy of the bovine female genital tract (from Budras and Habel, 2003)

The genital tract is attached to the dorsal abdominal wall and the sides of the pelvic canal by the broad ligaments (lig. latum uteri) on either side. The ligaments house the genital tracts' blood vessels and nerves. These ligaments can be divided into three parts that merge into each other without any obvious boundaries; the mesometrium, the mesovarium, and the mesosalpinx which is attached to the lateral side of the mesovarium. The mesometrium is attached laterally to the cervix and the curvatura minor of the uterus, which results in the uterine horns lying 'on top' of this ligament.

2.1.2 The ovaries

The bovine's almond shaped ovaries have an exocrine as well as an endocrine function, the production of resp. oocytes and steroid hormones. During fetal development oogonia are produced by mitotic multiplication of the primordial germ cells. Subsequently, millions of oocytes are formed during a first meiotic division which stops in the prophase. Most of these oocytes however will undergo atresia. At birth the ovaries already contain all oocytes a female calf will ever carry. Primordial follicles, from which up to 150.000 are present at birth, form when a layer of flat follicular or granulosa cells surrounds these oocytes (Hafez and Hafez, 2000). Before puberty these primordial follicles, can already develop into primary follicles, surrounded by one layer of cuboidal follicular cells, and secondary follicles, with multiple layers of follicular cells and small amounts of fluid between them. From this point on the zona pellucida, a non-cellular membrane between the oocyte and the granulosa cells, and the theca folliculi, that surrounds the whole follicle, will be present (Simoens, 2005; Van den Broeck, 2006).

The ovarian tissue can be divided into the cortex, which is the outer zone of the ovary and is surrounded by the germinal epithelium or surface epithelium, and the medulla, the central zone of the ovary. The outer zone of the cortex is called the tunica albuginea and gives the ovaries its white aspect. There is no distinct boundary between the cortex and the medulla, but follicles and CL's can be found in the cortex, whereas the medulla contains larger blood vessels, nerves and lymphaytics. The cortex also has a function of hormone-production. The blood vessels are important in maintaining this function. They do this by transporting hormones towards (FSH, LH, prostaglandins) and away from the ovaries (estradiol, progesterone) (Van den Broeck, 2006).

2.2 The estrous cycle of the cow

2.2.1 Puberty

In literature different definitions of puberty are used. Noakes (1997) defines the onset of puberty as the time when the ovaries start cycling on a regular basis. Ball and Peters (2004b) however, say that puberty starts when estrus first occurs, accompanied by ovulation.

Many factors influence the time of onset of puberty (in the female bovine) (Noakes, 1997; Ball and Peters, 2004b):

- Breed: in general dairy heifers reach puberty at an earlier age than beef heifers do.
- Body weight and nutrition: body weight seems to be more important than age in determining the time of onset of puberty. Slower growth delays the onset of puberty. In Holstein Friesians, puberty starts at 250-270 kg. Nutrition plays a pivotal role in the onset of puberty by mediating hormones such as insulin, insulin-like growth factors, and leptin.

- Season: although the evidence of seasonal influence is often conflicting, many studies have proven that the season of birth does play a role. Besides temperature and photoperiod, supplemental artificial lighting can quicken the onset of puberty.
- Social interaction: the presence of a bull can lower the age of puberty.
- Disease: this can slow the onset of puberty, particularly if growth is affected.
- Climate: warm climates have a negative influence on the onset of puberty.

Before puberty, there are already waves of follicular growth discernible on the ovaries, but all follicles will become atretic and thus no ovulation occurs (Noakes, 1997; Ball and Peters, 2004). This is because before puberty the neurons in the hypothalamus are too sensitive to the inhibitory effect of low levels of estradiol produced by the follicles. This causes a lack of Gonadotrophin releasing hormone (GnRH) and as a result also of LH. At the onset of puberty, as they mature, the neurons become less sensitive to this inhibitory effect and GnRH production will begin stimulating the secretion of FSH and LH. FSH stimulates follicular growth, while LH induces follicular maturation and ovulation (Noakes, 1997).

2.2.2 The ovarian cycle

Cows are poly-estrus with cycles averaging 21 days (70% within the range of 18 and 24 days). There is no cyclical activity before puberty, during pregnancy, and for a short period after calving (Noakes, 1997). The estrous cycle can be divided into different stages:

- Pro-estrus: Day 18-20 of the estrous cycle (Ball and Peters, 2004b). This is the stage before estrus. Typical in this stage are the regressing CL and the increased follicular growth. The dominant follicle can reach a diameter of 15 – 20 mm on average (Pieterse, 2008).The cow will start to show signs of her upcoming estrus, such as increased attempts to mount other cows.
- Estrus: This is considered to be day 21 or day 0 and lasts about 18 hours (15 hours for heifers) (De Kruif, 2009). The LH-peak which occurs 6 hours after the onset of estrus is followed by ovulation 24 hours later. The latter is considered as day 1 of the cycle and occurs approximately 12 hours after the end of estrus (Pieterse, 2008). Behavioral signs of estrus include:
    - Standing to be mounted. This is the most reliable sign.
    - (Head-)mounting other cows.
    - Elastic, mucous, clear, vulval discharge
    - Restlessness
    - Decreased milk yield
    - Bald areas on the tail-bone
- Met-estrus: Day 1-4. A corpus haemorrhagicum (CH) will develop which will later become a CL.

- Di-estrus: Day 5-18. The CL is the dominant structure. It will reach its maximal size (2-3 cm diameter) between day 8 and 17.

A different way to divide the estrous cycle in stages is according to follicular and luteal phase. The latter takes up most of the estrous cycle and is characterized by the forming or presence of an active, CL (d 1-17). The follicular phase is the period during which the CL regresses, a follicle develops into a pre-ovulatory follicle, and ovulation takes place. This lasts only 4 to 5 days (Hafez and Hafez, 2000b).

At every estrous cycle the LH surge will cause some of the secondary follicles to grow into tertiary or vesicular or antral follicles, which can be visualized at the surface of the ovary as small fluid containing blister-like structures. The fluid in between the follicular cells will increase in volume and flow together to form the antral cavity. The theca folliculi can now be divided into a theca externa which consists mostly of fibrous tissue, and a theca interna which is more cellular and contains many blood vessels (Ball and Peters, 2004a). A few of these vesicular follicles will develop into Graafian follicles (see Figure 2), the others will undergo atresia. The Graafian follicle is held in place in the follicular fluid by the cumulus oophorus, which has the shape of a peninsula. The follicular cells that lie directly against the zona pellucida are called the corona radiata. In a Graafian follicle the theca interna starts producing testosterone under the control of LH and FSH. Testosterone will be converted into estradiol by the granulosa cells and will then find its way into the blood. The highest concentrations of estradiol can be found at the beginning of behavioral estrus. In the pre-ovulatory period the vascular pattern of the ovaries undergoes major changes. This is made possible by the variations in the architecture of the vessels which allow adaptation of the blood supply (Hafez and Hafez, 2000). At ovulation, the oocyte continues the meiosis and will develop into an egg. Active ovaries will show different stages of follicles and/or CL's. Cows can have two, three, or occasionally four (Carrière et al., 2010) waves of follicular growth during one estrous cycle (see Figure 3). These are induced by FSH. From each wave one large dominant follicle of 12-15 mm will develop (Carrière et al., 2010). During the presence of a CL, progesterone prevents these follicles from achieving full maturation by negative feedback on LH (Noakes, 1997). Maturation and ovulation, usually of a single follicle, will occur when the CL regresses and progesterone concentrations are basal. This usually happens around day 16 or 17 of the estrous cycle. The first wave usually starts around day 2 or 3 of the cycle and the next wave 5-10 days later (De Kruif, 2009), but of course this depends on whether it is a single, double, or triple wave cycle. The surface of the ovaries is thus continually changing (Ball and Peters, 2004a).

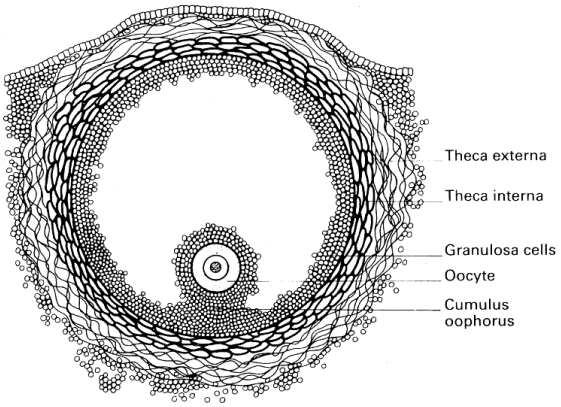

Figure 2. A Graafian follicle (from Noakes, 1997)

At ovulation, the oocyte, or actually the whole cumulus oophorus, is liberated from the Graafian follicle through a breach in the tunica albuginea. This breach is called the stigma. The Graafian follicle will change into a CH by filling the remains of the follicle with blood. After three days, a CL will form by re-absorption of the blood and the quick invasion of granulosa and theca interna cells into this cavity. LH plays a major role in this process. It can bind to receptors on the granulosal and thecal cells that have been developing before ovulation (Noakes, 1997). The granulosa and theca interna cells will become luteal cells, which will produce progesterone until day 16 or 17 of the cycle. A CL usually protrudes at the surface of the ovary, is yellow-brown, and in contrast to a follicle has a liver like consistency. In some CLs a cavity is present. This will be discussed later in this paper. A CL reaches its maximum size of 2.5-3 cm around day 7 or 8 of the estrous cycle and will often occupy more space than the remainder of the ovarian tissue. As a result of PG production by the endometrium, the CL starts to diminish in size around day 16 or 17 and eventually disappears (cyclic CL), a process that is called luteolysis (Noakes, 1997). In case a successful fertilization has taken place, it will remain present during the entire pregnancy (CL graviditatis). Remains of a CL will be visible for a long time as a white tissue-mass, the corpus albicans, or white body.

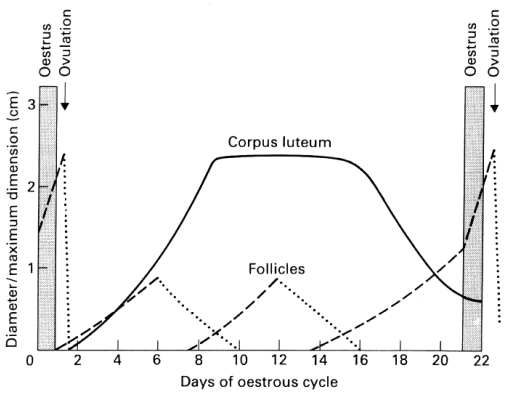

Figure 3. Growth and regression of the follicles and CL during the estrous cycle (this is an example of a cow with three waves of follicular growth) (from Noakes, 1997)

After luteolysis, when the negative feedback of progesterone disappears (see above), higher levels of GnRH will result in the production of FSH and LH. Follicles will start to grow, mature, and produce estrogen which will in turn cause an LH-surge and an ovulation (Pieterse, 2008). Sometimes however there is a lack of GnRH and therefore also LH which causes the follicles to not ovulate. As the follicle then may continue to grow, anovular follicle-like structures larger than 2,5 cm in diameter may persist. These are known as cysts. By definition, normal follicles are thus smaller than 2,5 cm in diameter. There are two types of pathological cysts: follicular and luteal. Follicular cysts have a thin wall (≤3 mm), luteal cysts have a thick wall (>3 mm) (De Kruif, 2009). Cows with pathological cysts are not cycling. Because the insufficiently-luteinized cysts do not produce enough progesterone, the uterus is not stimulated to start synthesizing PG and most of the affected cows will remain in anestrus.

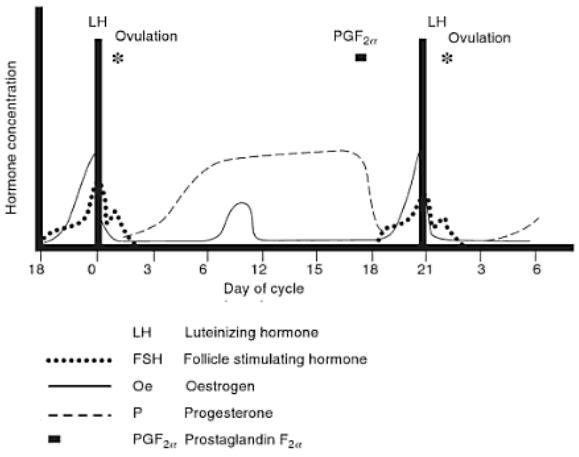

Figure 4. Hormonal changes during the estrous cycle (from Ball and Peters, 2004b)

Stress is thought to play a major role in the development of cysts (De Kruif, 2009).

2.3 Rectal palpation of the cycling cow

Before starting rectal palpation general external examination including an inspection of the perineum has to be done. This can be helpful to set the final diagnosis. There are a number of signs deserving our attention (De Kruif, 2009). Relaxation of the sacrotuberal ligaments, located on either side of the tale, can be seen in cows approaching their calving date and in cows with follicular cysts. By looking to the skin and tail eventual vaginal discharge can be observed. Discharge can be physiological, for example during estrus, or pathological, as in whites. Blood-tinged mucus can be seen on day two or three of the cycle in 50% of cows and 90% of yearlings (Pieterse, 2008). When inspecting the vulva attention should be paid to its position, discharge, swelling, and the measure of closing. The mucosa of the vulva (vulval vestibule) can also provide useful information, for example about the cycle stage. Normally this mucosa is pale, smooth and dull, but during estrus it is swollen and glossy. When inflamed, it is red (De Kruif, 2009).

When starting the actual rectal palpation, the feces should first be removed (Pieterse, 2008). After this, the different organs can be palpated (De Kruif, 2009). In order to do a perfect examination, the genital tract should be retracted into the pelvic canal. This can be done as follows (Pieterse, 2008): the first step is to put the cervix vertically. Then the uterus can be slightly pulled up at the bifurcation. After moving one's hand alongside the horn (left horn if left handed, right horn if right handed), the fingers can be hooked over the broad ligament and the thumb placed on top of the ligament, both as close to the uterus as possible. The ligament now seems to be positioned horizontally. While the thumb pushes the ligament down, the other fingers successively pull it forwards, upwards, en backwards, similar to accelerating a scooter. This will cause the uterus to start turning backwards. The last step is to move the fingers under the uterine horn (medially), in order to completely retract the uterine horn. The other horn will partially come up and can easily be retracted by making a scooping movement with the hand.

- Rectal palpation of the vagina is difficult, ineffective, and not routinely performed (Lefebvre and Gnemmi, 2010). In normal condition it feels like a slack thick tube, while in a puerperal cow it is soft and flexible. In case of perivaginitis, abscesses can be felt as harder 'cushions' on one or both sides of the vagina and in some cases crackling can be noticed (De Kruif, 2009).
- The point of orientation during rectal examination of the cow's genital tract is the cervix (De Kruif, 2009). Except for during parturition, the cervix is always firm due to the cartilage it contains (Pieterse, 2008). In a cycling cow, the cervix can be moved in all directions. In young cows the whole genital tract is located within the pelvic canal, in older cows the cervix lies near the front edge of the pelvis. Exceptionally no cervix (freemartins), or a double cervix can be present (De Kruif, 2009).
- Beyond the cervix, the uterine body and horns can be palpated. Attention should be paid to their size, symmetry, consistency, thickness of the wall, contents, adhesions, and tonus (De

Kruif, 2009). Due to high estrogen concentrations, the latter is significantly tenser around estrus. The uterine body feels softer than the cervix. The first few days post partum however, it feels doughy and wrinkly. When the uterine involution is delayed, the uterus is slack and smooth, or barely wrinkled. The size of the uterine horns depends on the parity of the cow. Asymmetry can be present in all cows which have calved. When noticed, it is therefore necessary to determine if it contains any content and if so, what is its nature (De Kruif, 2009). The uterine horns are bent in caudo-ventral (see above) direction. Sometimes it is necessary to retract them into the pelvic canal to be able to palpate them fully.

- The oviducts, because of their small diameter, can barely be felt in normal conditions (De Kruif, 2009). In case of inflammation or in case of hydro-salpynx, palpation of the oviducts is easier.
- The ovaries are normally located against the ventral part of the iliac shaft, at the same level as the bifurcation of the uterine horns (Lefebvre and Gnemmie, 2010). They need to be palpated for size, the presence of adhesions, and structures like CL's, follicles, and cysts. The ovaries can vary in size according to their activity and stage of the estrous cycle (Pieterse, 2008): a pea-size ovary is inactive, at the size of a broad bean it is normally active, at the size of a chestnut it is active and sometimes contains a young or old CL, at walnut-size it probably contains an active CL and if the ovary is bigger than a mandarin or a small orange it is usually abnormal (Pieterse, 2008). The largest ovary is usually most determinative to estimate the current stage of the cycle.

  A follicle can be palpated as a smooth and fluctuating structure (De Kruif, 2009). Cysts can be palpated as large follicle-like structures. It is, however, hard to distinguish between follicular and luteal cysts by rectal palpation. Follicular cysts have a thin wall, are fluctuant and likely to rupture during manipulation. Luteal cysts have a thicker wall which makes them feel firmer (Hanzen et al., 2000). A fresh ovulation site can be palpated as a depression, but this may be difficult to recognize. A CH is also rather difficult to recognize. It is a soft, crepitant structure, less than 1 cm high. It is smaller than a mature follicle or a mature CL and its surface is less firm and smooth than that of a follicle (Hanzen et al., 2000). A cyclic CL is firm, does not fluctuate unless it contains a cystic structure, and can have a crown or ovulation papilla that protrudes from the surface of the ovary (De Kruif, 2009). The appearance of the CL however, varies during the estrous cycle (see Table 1). The older it gets, the harder it will be. The regressing CL can be palpated until a few days after the next ovulation (Hanzen et al., 2000). Determining the age of the CL is needed in order to start a treatment with prostaglandin or synchronization of ovulation (OvSynch).

Various authors have carried out studies to determine the sensitivity and specificity of rectal palpation in order to estimate the stage of the cycle. This will be discussed later.

Table 1. Findings on rectal palpation during the estrous cycle (according to Pieterse, 2008)

	CL	Follicles	Uterus
D0	Old, small and hard	Small follicles and 1-2 bigger ones (15-20mm)	Very contractile, tonus ++, elastic vulval mucus
D1	Idem	Ovulation	Tonus ++
D2	CH (small, soft)	Ovulation place/small follicles	Tonus +, (bleeding)
D3	Idem	Idem	Tonus +, (bleeding)
D4	Growing, palpable		Tonus ±
D5	Idem		Tonus ±
D6	Idem		Tonus ±
D7	Idem		Tonus ±
D8	Idem		Tonus ±
D9	Mid-cycle max. CL 25-35 mm	Often bigger, growing follicles from 1^{st} or 2^{nd} follicular wave	Tonus ±/+
D10	Mid-cycle max. CL 25-35 mm	Often bigger, growing follicles from 1^{st} or 2^{nd} follicular wave	Tonus ±/+
D11	Idem	Idem	Tonus +
D12	Idem	Idem	Tonus +
D13	Idem		Tonus ±
D14	Slowly becoming firmer		Tonus ±
D15	Idem		Tonus ±
D16	Idem		Tonus ±
D17	Regressing and becoming harder		Tonus ±/+, watery mucus
D18	Idem		Tonus +, smear of mucus
D19	Idem	Evt. palpable follicle >10 mm	Tonus +, longer smear of mucus
D20	Idem	Idem	Tonus +/++, elastic mucus till the ground
D21	Idem	Easily palpable, big pre-ovulatory follicle <25 mm	Tonus ++, elastic mucus till the ground

2.4 Ultrasonography

2.4.1 General

Although ultrasonography was first introduced in veterinary medicine in 1971 (Gielen et al., 2008), it has only become indispensible in bovine veterinary practice during the previous couple of years. Not only can it be used to verify the rectal examination, it is a good tool to make a more reliable diagnosis and to determine a more effective treatment. Ultrasonography is based on the direction and reflection of sound waves. The sound waves are sent into the tissue by a transducer which contains crystals which vibrate when electricity is passed through them. The frequency of the sound waves can vary from 1-20 MHz. Higher frequencies produce better resolutions, but do not penetrate as far into the tissue. The waves will reflect on the boundary of two tissues, depending on the speed of the waves and the difference in the tissues' densities. The reflected waves are received by the transducer which again causes a vibration of the crystals. The electricity produced is then converted into an image. Each point on the image lies at a distance proportional to the time between sending and receiving the wave, and each point is shaded more white ((hyper)echogenic) or black (anechogenic or hypoechogenic) according to the strength of the received wave. An object is hyperechogenic when it strongly reflects the waves, e.g. bone, and anechogenic when it does not reflect the waves, e.g. water and air.

In radiology, the most common type of ultrasonographer is the B (Brightness)-mode, which produces a two-dimensional image that can be described as a 'cut' through the tissue. Depending on the kind of transducer used, this two-dimensional image can be either rectangular (with a linear transducer) or cone-shaped (with a convex transducer). The linear transducer is better for superficial tissues, while the convex is better for deep structures.

2.4.2 The cycling bovine genital tract

In order to visualize the genital tract transrectal ultrasonography is preferred. Before starting, the feces need to be removed from the rectum. After manually localizing the ovaries and eventually retracting the genital tract into the pelvic canal, the transducer can be advanced into the rectum and placed against the structure to be visualized.

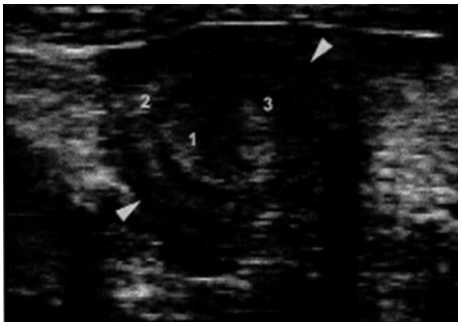

Figure 5. Ultrasonographic image of a transverse section of the uterine horn (probe 7.5 MHz; depth 5 cm). 1: Endometrium; 2: Myometrium; 3: Vascular portion of the uterus; Arrowheads: Edge of the uterus (from DesCôteaux et al., 2010)

The ultrasonographic image of the uterus typically shows the different echotextures of the different layers (see Figure 5). Peripherically the uterine horns produce a hyperechogenic signal. Under this the blood vessels and the myometrium can be visualized as a faint echogenic line. The endometrium is an echogenic structure containing dark and bright signals. In an empty uterus, the dorsal and ventral endometrium will join and appear as a bright line (Lefebvre

and Gnemmi, 2010). During pro-estrus and estrus, some fluid can be present in the uterus. In the presence of a CL however, fluid is usually pathological (Colazo et al., 2010). Ultrasonography is a very accurate method to identify ovarian structures. Inactive ovaries do not contain any structures and have a quite uniform echogenicity. Because of this, they can sometimes be harder to find than active ovaries. The ovarian stroma is echogenic. Follicles can easily be observed as dark anechogenic spherical structures of various sizes (due to the fluid they contain). Their shape can become irregular in the presence of adjacent follicles or a CL that is compressing the follicle (Pierson and Ginther, 1988), but also because of transducer pressure (Lefebvre and Gnemmi, 2010). At their distal zone a hyperechogenic border can usually be seen (Hanzen et al., 2000). Except for the first few days of the cycle, follicles larger than 8 mm are always present (Carrière et al., 2010). Follicles can reach a size up to 2,5 cm in diameter. The real diameter of a follicle is however often underestimated by 2-3 mm, because the ultrasound only shows the follicular cavity and not the wall (Hanzen et al., 2000). The borderline between the follicular wall and the ovarian stroma is only identifiable in large preovulatory follicles. The line between the follicular wall and the antrum however is always well defined and smooth (Lefebvre and Gnemmi, 2010). Follicle-like structures larger than 2,5 cm are cysts (see under 2.2.2). Ultrasonography can help to distinguish between follicular and luteal cysts and if a clear difference in thickness of the wall exists identification is greatly simplified. Leidl et al. (1979) have noted however, that transitional forms occur, and that about 34% of follicular cysts have some degree of luteinization within their wall. It is also important to distinguish follicles from blood vessels. This can be done by moving the transducer in the direction to make a longitudinal section instead of a cross section. When looking at a follicle, the initial spherical image will become smaller and later disappears. When looking at a blood vessel, the initial spherical image will become elongated (Carrière et al., 2010). The size of the largest follicle cannot be used to determine the stage of the estrous cycle because of the different follicular waves throughout the cycle. It is better to rely on the appearance of the CL. As mentioned before, determining the age of the CL is crucial to decide when to start a treatment with prostaglandin or synchronization (OvSynch). A CH is a poorly defined, irregular, greyish-black structure with several echogenic spots, all within the contour of the ovary (Pieterse et al., 1990; Hanzen et al., 2000). Occasionally the crown-like ovulatory papilla can be seen (Ribadu et al., 1994). Ovulation can also indirectly be detected by the disappearance of a pre-ovulatory follicle, or it can be presumed to have occurred when there is a small CL and (usually) an absence of follicles (Colazo et al., 2010). A CL increases in echogenicity during diestrus (Hanzen et al., 2000), but because of its vascularization it is hypoechogenic compared with the ovarian stroma (Carrière et al., 2010). It can be seen as a greyish, echogenic structure with a demarcation line between it and the ovarian stroma (Pieterse et al., 1990; Hanzen et al., 2000). A regressing CL becomes more hyperechogenic and has a faint demarcation line due to the little difference in echogenicity between it and the ovarian stroma (Pieterse et al., 1990). Corpora albicantia can, in general, not be identified due to their size and their echogenic resemblance to the ovarian stroma (Lefebvre and Gnemmi, 2010).

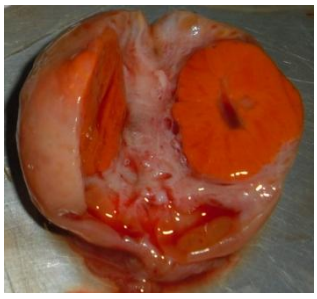

Figure 6. A cystic CL

CL's with a fluid-filled central cavity (cystic CL or CCL) can sometimes be observed (see Figure 6). Nearly 80% of heifers have one (Kastelic et al., 1990[a,b]). The size of this cavity can vary from 2-22 mm (Hanzen et al., 2000), and they are largest from days 6.5 to 8 of the estrous cycle (Kastelic et al., 1990[b]). In appearance a CCL's cavity resembles that of a follicle's (Kähn and Leidl, 1989), but usually it is less regular, surrounded by luteal tissue (Pierson and Ginther, 1987), and occasionally echogenic fibrin strands can be present within the cavity (Carrière et al., 2010). The CCL is a physiological structure. Its presence and size do not influence the ovarian cycle, progesterone levels, or the potential of an animal to become pregnant (Kastelic et al., 1990[a]; Ribadu et al., 1994). They usually develop when the CL is still young (Max et al., 1997), and are last seen 9.3, 11.1 and 17.4 days after ovulation for respectively small, medium, and large cavities (Kastelic et al., 1990[b]). A cow with a CCL in her current cycle does not have a higher chance of getting a CCL in her next cycle than any other cow (Kastelic et al., 1990[b]). CCLs can sometimes even be observed in early stages of pregnancy (Kastelic et al., 1990[b]; Colazo et al., 2010).

It can sometimes be hard to differentiate between a CCL and a luteal cyst, e.g. a small luteal cyst with a relatively thick wall could be mistaken for a large CCL. Here are some points to keep in mind (Kähn and Leidl, 1989):

- A CCL is usually ≤3 cm in diameter and the surrounding luteal tissue is about 5-10 mm thick.
- A CCL is, just like any CL, usually oval shaped and rarely round.
- The cavity of the CCL is normally homogeneous and near-black, whereas luteal cysts frequently present reflections. The cavity of a CCL can occasionally show echogenic fibrin strands (Carrière et al., 2010). The cavity of a luteal cyst often shows trabeculae (Hanzen et al., 2000; Pieterse, 2008).
- A CCL will undergo cyclic development like regression of the cavity, whereas a cow with luteal cysts is not cycling.

2.5 Progesterone levels during the estrous cycle

Progesterone is produced by the luteinized cells from the CL. It largely suppresses gonadotrophin release by negative feedback (Noakes, 1997) and is used in a number of studies (Ireland et al., 1980; Pieterse et al., 1990; Ribadu et al., 1994; Battocchio et al., 1999; McDougall and Rhodes, 1999) to determine a cow's stage of estrous cycle. Ireland et al. (1980) took blood samples of 146 heifers, which were observed for estrus twice a day. Average progesterone concentrations (in ng/ml) were: 1.5 from days 1-4, 6.9 from days 5-10, 7.8 from days 11-17, and 1.2 from days 18-20. Battocchio et al. (1999) found that, when progesterone concentrations were less than 1 ng/mL, no CL was present. When concentrations were between 1 and 4 ng/mL, an evolving CL was present, and when progesterone concentrations exceeded 4 ng/mL, a CL was classified as being mid-cycle. These same values were used by Veronesi et al. (2002).

2.6 Research by others

In the past, different authors have tried to determine the accuracy of rectal palpation and of ultrasonography of the reproductive tract in cows. Below, some of these studies will be discussed.

2.6.1 Pieterse et al. (1990)

A comparison was made between rectal palpation and transvaginal ultrasonography to detect CLs and follicles in 59 cows of unknown reproductive history. Rectal palpation was followed by ultrasonography, taking a blood sample and dissection of the ovaries after slaughter. During rectal palpation follicles of more than 5 mm in diameter were counted and classified into groups according to their size: 5-10 mm, 10-15 mm and more than 15 mm. CLs were also divided into different categories: young CL (days 1-4), mid-cycle (days 5-16) and old (days 17-20). The age of the CLs was estimated by their diameter and firmness. A young CL was defined as a small, soft structure on the sometimes irregular surface of the ovary. A mid-cycle CL would sometimes protrude above the surface of the ovary and was more defined than a young CL. An old CL would protrude from the surface of the ovary less than a mid-cycle CL, but it was more compact and almost as firm as the ovary itself. Follicles could be palpated as smooth and fluctuating structures on the surface of the ovary.

After an interval of at least an hour, the same animals were examined by the same person by transvaginal ultrasonography, without the records of the rectal palpation being available. The follicles and CLs were again classified according to the categories mentioned before. The definition for a young CL was a poorly defined, irregular, greyish-black structure with echogenic spots, all within the contour of 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. The demarcation line was faint in an old CL. Follicles were described as black, circumscribed areas, clearly contrasting with the ovarian stroma. After ultrasonography a jugular, heparinised blood sample was taken. These samples were later analysed for progesterone by radioimmunoassay. The mean concentrations during each of the three stages of CL development were later used to confirm their morphological classification after dissection.

Table 2. The presence and age of CLs diagnosed by rectal palpation and ultrasonography compared with inspection after slaughter (n = 118 ovaries) (from Pieterse et al., 1990)

Corpora lutea identified by dissection	n	Corpora lutea identified by rectal palpation				Corpora lutea identified by ultrasonography			
		Young	Mid-cycle-	Old	None	Young	Mid–cycle	Old	None
Young	**9**	**4**	3	1	1	**3**	2	0	4
Mid-cycle	**36**	0	**30**	4	2	3	**29**	4	0
Old	**11**	1	2	**7**	1	1	2	**4**	4
None	**62**	0	6	3	**53**	0	1	4	**57**

The cows were slaughtered on the same day and the 118 ovaries were dissected. The ages of the CLs were judged by morphological criteria (histology) and the follicles were counted and measured under the microscope.

Neither rectal palpation nor ultrasonography proved a good method for detecting young CLs (Table 2 and Table 3), but rectal palpation had a much higher positive predictive value. For the detection of mid-cycle CLs both techniques were as accurate, but the positive predictive value was higher for ultrasonography. Rectal palpation seemed to be better for the detection of old CLs. Rectal palpation was better to detect young, mid-cylce and old CLs together, but the difference with ultrasonography was not significant: for rectal palpation values of 73.2% and 67.2% were found for sensitivity and predictive value respectively, whereas for ultrasonography these were 64.3% and 67.9%.

Both techniques were able to correctly diagnose the absence of a CL. Ultrasonography, however, incorrectly diagnosed four old CLs and one mid-cycle CL. With rectal palpation, six mid-cycle CLs were diagnosed when in reality no CLs were present. These incorrect diagnoses were caused by the presence of luteinised follicles, two large follicles (> 15 mm) on one ovary, and by misinterpreting the ovarian stroma.

Table 3. Sensitivity (%) and predictive value (%) of rectal palpation and ultrasonography for detecting CLs of different ages (from Pieterse et al., 1990)

Technique	Evaluation of results	Corpora lutea: Young (9)	Mid-cycle (36)	Old (11)	None (62)
Rectal palpation	Sensitivity	44·4	83·3	63·6	85·5
	Predictive value	80·0	73·2	46·7	93·0
Ultra-sonography	Sensitivity	33·3	80·6	36·4	91·9
	Predictive value	42·9	85·3	33·3	87·7

2.6.2 Grygar et al. (1992)

In this study a comparison was made between the accuracy of rectal palpation and transrectal ultrasonography to diagnose both physiological and pathological conditions in bovine ovaries. The ovaries of 33 non-pregnant Czech cows, with unknown reproductive history, were examined by rectal palpation by two examiners and by ultrasonography by one examiner. In about half of the cows rectal palpation was done first, then ultrasonography. The other half of the cows were examined in the opposite order. Approximately 20 hours after palpation and ultrasonography the cows were slaughtered and the ovaries cross-sected. In all three examinations follicles and CLs were divided into classes according to their size. For follicles 5 classes were used: 2-5 mm, 6-10 mm, 11-15 mm, 16-20 mm, and 21-25 mm in diameter. Follicle-like structures larger than 25 mm were classified as follicular or luteal cysts. For CLs the examiners worked with 3 groups: 6-10 mm, 11-20 mm, and more than 20

Table 4. Comparison of rectal palpation, ultrasonography, and inspection after slaughter (from Grygar et al., 1992)

Examination method	Follicles (mm)					Follicular cysts (mm)	Corpora lutea (mm)			Luteal cysts
	2–5	6–10	11–15	16–20	21–25	>25	6–10	11–20	>20	
Rectal palpation (examiner 1)	86 (+6)	16 (+19)	13 (+15)	2 (+2)	1 (+1)	2	9 (+13)	7 (+6)	13 (+3)	(+2)
Rectal palpation (examiner 2)	5	15 (+11)	5 (+4)	3 (+1)	1	2	5 (+4)	5 (+5)	10 (+1)	(+1)
Ultrasonography	134 (+3)	32 (+2)	22 (+1)	7	1	2	8 (+1)	9	17	0
	261	36	22	8	1	2	14	9	17	0

Note: The numbers of false positive diagnoses are in brackets

mm. For CLs, age and morphology or echogenicity were taken into account as well. In case of a cavity present within a CL, attention was paid to its features.

The number of follicles and CLs counted during each examination are listed in Table 4. The findings resulting in a false positive diagnosis were either incorrectly diagnosed or incorrectly classified by the examiners. The findings after slaughter can be found in the bottom line of the table. Note the differences between ultrasonography and rectal palpation, and within the latter also between examiner 1 and 2, in the detection of follicles from 2-5 mm in diameter. The accuracy for the different examination methods are calculated for the different size classes and listed in Table 5. Typical for the smallest follicles is the high predictive value and the low sensitivity. For the follicles 11-25 mm in size reliability of ultrasonography was very high. Except for CLs 6-10 mm in diameter, ultrasonography had better results in detecting ovarian structures than rectal palpation.

Table 5. The sensitivity (C) and positive predictive value (PPH) of rectal palpation and ultrasonography based on inspection of the ovaries after slaughter (from Grygar et al., 1992)

Examination method		Follicles (mm)					Corpora lutea (mm)		
		2–5	6–10	11–15	16–20	21–25	6–10	11–20	>20
Rectal palpation (examiner 1)	C%	33 0	44 4	59.1	25.0	100	64.3	77.8	76.5
	PPH%	93 5	45 7	46.4	50.0	50.0	40.9	53.8	81.3
Rectal palpation (examiner 2)	C%	1.9	41.7	22.7	37.5	100	35.7	55.6	58.8
	PPH%	100	57 7	55.6	75.0	100	55.6	50.0	90.9
Ultrasonography	C%	51 3	88 9	100	87.5	100	57.1	100	100
	PPH%	97 8	94 1	95.7	100	100	88.9	100	100

After slaughtering 14 CLs (out of 40) were found to contain a central cavity. Examiner 1 detected 12 CLs with a cavity, but only 5 of those were correctly diagnosed (sensitivity of 41.7%). His predictive value was relatively low as well. Examiner 2 also detected 12 CLs with a cavity, but now only 1 was correct. His predictive value was 100%, because he correctly referred to the 15 mm cavity as being a large one. Ultrasonography correctly detected all cavities filled with clear liquid (10), but it failed to detect any cavities filled with blood clots (4). 3 Out of these 4 however, had dimensions on the threshold for detection (2-3 mm). Ultrasonography had a predictive value of 100% for CLs with cavities.

2.6.3 Ireland et al. (1980)

In 180 Hereford heifers of unknown ages, the stage of estrous cycle was estimated by inspection of the CLs after slaughter. This was done by an examiner with no prior knowledge of their reproductive history. In order to obtain the accuracy of inspection of CLs to determine the cycle stage, these results were compared with the findings of another examiner, who observed the estrus in these animals twice a day (from 7-8 am and 4-5 pm) to obtain the actual day of the estrous cycle. At slaughter, jugular blood samples on heparin were collected and kept on ice until centrifugation immediately after arrival at the laboratory. Plasma was frozen until analyzed for progesterone by radioimmunoassay.

CLs were classified into four groups by four easily identifiable changes in appearance during the estrous cycle (see Table 6). Stage 1 represented the time from ovulation until the time when

Table 6. Classification of CLs during the estrous cycle (from Ireland et al., 1980)

Characteristics	Stages of estrous cycle[a]			
	I	II	III	IV
Appearance of corpus luteum				
External	red, recently ovulated, point of rupture not covered over by epithelium (see arrow in I)	point of rupture covered over, apex of corpus luteum red or brown (see left ovary in II)	tan or orange	light yellow to white
Internal	red, occasionally filled with blood, cells loosely organized (see right ovary in I)	red or brown at apex only, remainder of corpus luteum is orange (see right ovary in II)	orange	orange to yellow (see arrow in IV)
Diameter	.5–1.5 cm	1.6–2 cm	1.6–2 cm	<1 cm
Vasculature on surface of corpus luteum	not visible	generally limited to periphery (see arrow plate II)	same as in II but it will cover apex of corpus luteum late in this stage (see arrow in III)	not visible
Follicles >10 mm in diameter	absent	present (not shown)	may be absent or present	present (see left ovary in IV)

epithelium grows over the rupture point, forming the apex of a new CL. Stage 2 is marked by a fully formed CL with blood vessels visible around its periphery. When bisected, its apex is red or brown while the rest is orange or yellow. During stage 3 the entire CL is bright orange or yellow. Blood vessels are visible over the apex of the CL late in this stage. Typical for stage 4 is the regressed CL (the authors used 20 days as being the average of the heifer's estrous cycle). The vascular network on its surface had disappeared and the ovary usually contained at least one large follicle (10 mm or more).

Of 180 heifers, in 43 (23%) estrus was never observed, whereas in 89 (50%) estrus was observed at least once but with irregular intervals. Estrus may have been missed in many of these, possibly also due to overcrowding and slippery floors. The remaining 48 animals (27%) had at least one regular estrus interval (17-25 days). All heifers were slaughtered, the last group within 21 days of estrus. 19 Of the 43 animals that were never observed as being in estrus contained neither CLs nor corpora albicantia. These heifers were probably prepubertal. 146 Animals had normal CLs and were divided into one of the four stages.

Because the length of each of the four stages was unknown at the start of this experiment, it was estimated by multiplying the percentage of the total number of animals in each stage by the average length of the heifer's estrous cycle. This can be seen in Table 7.

Table 7. Relationship between the stage of estrous cycle and estimated days of the estrous cycle (from Ireland et al., 1980)

Stage of estrous cycle based on appearance of corpus luteum	Number of heifers	Percent of total	Estimated length of each stage of estrous cycle (days)	Estimated days of estrous cycle[a]
I	30	20	4	1–4
II	42	29	6	5–10
III	52	36	7	11–17
IV	22	15	3	18–20
Total	146	100	20	20

Note: day of ovulation = day 1 and day of estrus = day 20

The accuracy of inspection of the ovaries after slaughter was determined by comparing the results from the two examiners (Table 8), but only for the 48 heifers with accurate estrus data. A correlation of .81 (P<.01) was found between estimated and actual day of the estrous cycle, and 85% of the heifers (41 of 48) were placed in the correct stage or ±1 day of the correct stage.

Table 8. Frequency distribution of actual and estimated days of estrous cycle (from Ireland et al., 1980)

Stages and estimated days of the estrous cycle	Actual day of estrous cycle																				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Overall
I (1–4)	1		2		4																7
II (5–10)			1	2	1	1		2	2	1	1	1					1	1			14
III (11–17)								1		3	1	1	2	2	1	1	2	5	1		20
IV (18–20)																	2	1	3	1	7
Overall	1	0	3	2	5	1	0	3	2	4	2	2	2	2	1	1	5	7	4	1	48

Average progesterone concentrations (in ng/ml) of the 146 heifers were: 1.5 in stage 1, 6.9 in stage 2, 7.8 in stage 3, and 1.2 in stage 4. No differences in concentrations were found between heifers with regular and heifers with irregular estrus intervals.

Ireland et al. (1980) concluded that inspection of CLs after slaughter can be used to estimate stages of the estrous cycle in Hereford heifers.

2.6.4 McDougall and Rhodes (1999)

The level of agreement between rectal palpation, ultrasonography, and plasma progesterone concentration to detect a CL was determined in 457 post-partum dairy cows (Jersey, Friesian or crossbred) not seen in estrus. All cows had calved more than 21 days ago. Hundred sixty two of these cows were examined by rectal palpation and transrectal ultrasonography, both by the same examiner.

For rectal palpation a CL was defined as a firm structure with or without a protruding 'button'. For ultrasonography, a CL was identified as a hypoechogenic heterogeneous mass, often with a non-echogenic antral cavity. In 103 of these cows, blood samples were taken from the coccygeal vein to measure progesterone concentrations. Evacuated tubes containing lithium heparin preservatives were used. Samples were placed on ice and centrifuged within four hours, after which plasma was frozen until analyzed by radioimmunoassay. The remaining 295 cows were examined using ultrasonography alone. The stage of estrous cycle could not be determined in this study, as the cows had not been detected in estrus before examination, and no progesterone measurements had been done either.

Before starting this experiment however, the association between progesterone concentrations and the detection of CLs by ultrasonography still had to be determined. This was done by daily examination of eight 2-year-old Friesian cows from approximately 37 days after calving until the subsequent ovulation. The CLs were measured and blood samples were collected as described above (Figure 7). Ultrasonography detected more CHs or CLs than when the progesterone concentration was higher than 1 ng/ml. Ultrasonography detected a CH or CL in all cows from day 2-20 (85%) of the cycle, and is thus a better technique to classify a cow as cycling than a single progesterone measurement. For subsequent comparisons, ultrasonography was used as the golden standard.

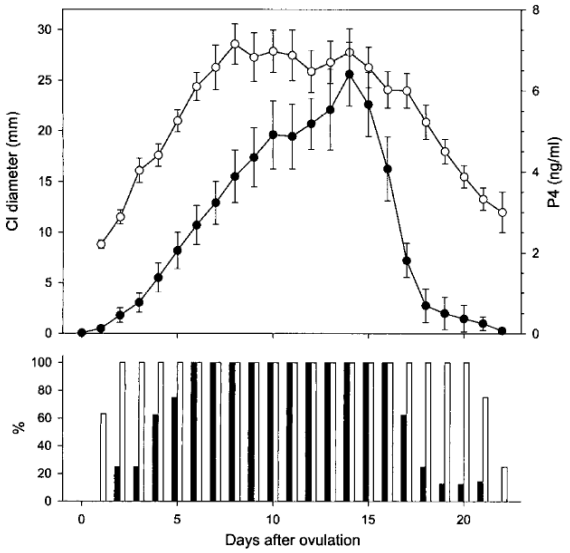

Figure 7. Top: mean CL diameter (○) and progesterone concentrations (●) for eight cows measured daily during a complete estrous cycle. Bottom: The percentage of cows with a CL detected (open bar) or with plasma progesterone > 1.0 ng/ml (filled bar) on each day of the estrus cycle (from McDougall and Rhodes, 1999)

Of the group of 162 cows, two were diagnosed as having follicular cysts (large, anechogenic structures >40 mm in diameter), in 92 no CL was detected, and in 44 cows a CL was detected by both palpation and ultrasonography (see Table 9). 20 CLs were detected by ultrasonography alone, and four CLs were detected by palpation but not by ultrasonography. There was a significant (p<.001) and high level of agreement (kappa =.67) between the two techniques. With ultrasounds used as the golden standard, rectal palpation would have a sensitivity and specificity of respectively 68.7% and 95.8%, and a positive and negative predictive value of 91.7% and 82.1%.

Table 9. Number of cows where a CL was found by palpation and/ or ultrasonography (from McDougall and Rhodes, 1999)

		Ultrasound		
		CL present	CL absent	Total
Manual palpation	CL present	44	4	48
	CL absent	20	92	112
Total		64	96	160

The progesterone cut-off value at which sensitivity and specificity were optimized for detection of a CL with ultrasonography or palpation was 0.675 ng/ml. With this cut-off, the sensitivity, specificity, positive and negative predictive value for progesterone compared to ultrasonography were 82.6%, 91.2%, 88.3% and 86.6% respectively. The level of agreement (kappa) at this cut-off was .74 ($p<.001$), while it was only .67 for ultrasonography and palpation.

2.6.5 Ribadu et al. (1994)

The accuracy for determining the presence and age of a CL by rectal palpation and transrectal ultrasonography was determined in 34 Holstein Friesian cows of unknown reproductive history. The presence of follicles larger than 10mm in diameter was also recorded. All examinations were done by the same person and during each of the examinations the CLs were classified into three stages: developing (days 1-4), mid-cycle (days 5-6) or regressing days (17-21). At rectal palpation a developing CL was a small, soft structure on the surface of the ovary, a mid-cycle CL was more defined and would sometimes protrude from the surface of the ovary, and a regressing CL was a more compact structure which was almost as firm as the ovarian stroma and protruded from the surface less than at mid-cycle. At ultrasonography the same characteristics as Pieterse et al. (1990) were used to divide the CLs into stages. Also the maximum diameter of the CL was measured. After slaughter CLs and large follicles were measured and the mid-cycle CLs were weighed. A blood sample from the tail vein was taken before slaughter. Plasma progesterone concentration was used as the golden standard.

Table 10. Classification based on inspection after dissection, rectal palpation and ultrasonography (from Ribadu et al., 1994)

Stage of corpus luteum	Dissection	Classification by Palpation	Ultrasound
Developing	0	0	0
Mid-cycle	20*	17	19*
Regressing	3	0	1

Note: * Three double CLs were each counted as one unit

The results from inspection after slaughter, rectal palpation and ultrasonography are shown in Table 11. Classification based on inspections of the ovaries after slaughter and plasma progesterone concentrations were completely the same: of the 34 cows, three had regressing CLs and 11 had no CLs. No developing CL was found by any of the three methods. Ultrasonography correctly identified three double CLs and two CLs with cavities. Rectal palpation wrongly classified one regressing CL as a mid-cycle CL, and ultrasonography only identified one of the three regressing CLs. Only 28% of follicles larger than 10 mm were detected by palpation, whereas ultrasonography

Table 11. Sensitivity, specificity and positive predictive value of rectal palpation and ultrasonography for identifying mid-cycle CLs (from Ribadu et al., 1994)

	CL found	CL not found
Palpation per rectum		
CL present	17 (a)	2 (b)
CL not present	3 (c)	46 (d)
Sensitivity = a/(a+c) x 100 = 17/20 x 100 = 85%		
Specificity = d/(b+d) x 100 = 46/48 x 100 = 95·8%		
Positive predictive value = a/(a+b) x 100 = 17/19 x 100 = 89·5%		
Ultrasonography		
CL present	19 (a)	0 (b)
CL not present	1 (c)	48 (d)
Sensitivity = 19/20 x 100 = 95%		
Specificity = 48/48 x 100 = 100%		
Positive predictive value = 19/19 x 100 = 100%		

was able to identify 76% of those. As discussed above, Pieterse et al. (1990) detected respectively 71% and 95% of those. Both ultrasound and rectal palpation had a high sensitivity for mid-cycle CLs, but compared with rectal palpation, ultrasonography had higher sensitivity, specificity and positive predictive values in this category (Table 11). A linear relationship was seen between the weight of mid-cycle CLs and plasma progesterone concentration. A high correlation was seen between the diameter of a CL determined with ultrasound and the progesterone concentration, although the drop in progesterone concentration during the last days of the cycle is not accompanied by an equal decline in diameter.

2.6.6 Dawson (1975)

180 Cows were examined by rectal palpation and after slaughtering their ovaries were inspected and dissected for detection of ovarian structures. Eighty five cows were slaughtered within 24 hours after examination and 95 within 48 hours. No attention was paid to follicles smaller than 7.5 mm and CLs smaller than 10 mm in diameter. Cysts were defined as follicular structures more than 20 mm in diameter. Classification was purely based on the detected ovarian structures.

Table 12. Accuracy of rectal palpation compared to inspection after slaughter (from Dawson, 1975)

1	2	3	4	5	6	7
	Total number	+	±	Sum of columns 3 and 4	—	Per cent —
Individual ovaries	359*	250	48	298	61	17
Individual cows	180	88	30	118	62	34

42 Cows did not appear to be cycling and in the remaining 138 cows ovarian function seemed normal. Table 12 shows the accuracy of rectal palpation compared to inspection after slaughter for all 180 cows; '+' means a correct diagnosis, '-' results are incorrect, and '±' denotes minor errors. Eighty three % of the ovaries had been assessed correctly, but this was only the case for 67% of the cows. In the '±' category, which contains 13% of the ovaries, the main structure (CL, large follicle or cyst) had been diagnosed correctly, but smaller structures of the same or different types had not been palpated. However, a small error like this in one of the ovaries of a cow would not necessarily lead to a wrong estimate of a cow's reproductive status. If the two ovaries of a single cow had been divided into different categories, the individual cow would be diagnosed in the worst one of those two categories. The errors made in the 61 ovaries categorized as '-' are specified in Table 13. In more than half of these ovaries errors were due to failure to detect follicles or small cysts in ovaries otherwise empty. The structures diagnosed, but not confirmed at autopsy, were one four-day old CL, one CL 20 mm in diameter, and two ovaries with follicles up to 8 mm in diameter.

Table 13. Incorrect diagnoses of ovaries (from Dawson, 1975)

	Number	Per cent
Ovaries not found	2	3·2
*Follicles not detected in otherwise empty ovaries	27	44·4
*Cyst(s) not detected	4	6·6
Corpora lutea not detected	9	14·7
Luteal remnants not detected	3	4·9
Corpora lutea diagnosed as cysts or follicles	6	9·8
Follicles/cysts diagnosed as corpora lutea	5	8·2
Corpus luteum and cyst not detected	1	1·6
Structures diagnosed, not confirmed at autopsy	4	6·6
Total	61	100·0

Rectal palpation correctly diagnosed 89% (125 out of 141) of the ovaries containing CLs, but in 13 of those ovaries other structures were missed (Table 14). In six ovaries, CLs were wrongly identified during rectal palpation as cysts or follicles. In 10 ovaries, CLs were missed by rectal palpation: these CLs were mostly less than 20 mm in diameter. Among the ovaries correctly identified as containing CLs, 11 each contained a CL with a central cavity greater than 10 mm in diameter. Of these cystic CLs, eight were recognized as such by rectal palpation, but three were identified as a regular CL. Two ovaries with a cystic CL were wrongly identified as containing a cyst. 80% of cysts were correctly identified, but with 11 of those minor errors were seen. In three ovaries cysts were wrongly classified as CLs, and in five ovaries cysts of up to 25 mm were not detected.

Table 14. Accuracy of rectal palpation for identification of CLs and cysts (from Dawson, 1975)

Total of ovaries	Post mortem finding	*In vivo* diagnosis			
		+	±	— Wrongly identified	— Not detected
141	Corpus luteum	112	13	6	10
41	Cyst(s)	22	11	3	5

2.6.7 Max et al. (1997)

41 Heifers were examined by rectal palpation and transrectal ultrasonography between days 12 and 15 of the estrous cycle for detection of ovarian structures. According to these researchers, CLs will have obtained a typical morphology at this time of the cycle: they will protrude from the ovarian surface and will be easy to distinguish from the ovarian tissue. All animals had been given two PG injections, 14 days apart, to synchronize the estrus, and 24 animals had also been given an injection with PMSG, 12 days after the first PG injection. The results were compared with inspection of the ovaries after slaughter, which took place right after the two initial examinations. The ovaries were divided into four groups: group I contained ovaries without a CL, ovaries with just one CL belonged in group II, ovaries with more than one CL in group III, and ovaries with both follicles and CLs in group IV.

Table 15. Ovarian structures diagnosed by each method (according to Max et al., 1997)

Group	Ultrasonography	Rectal palpation
I (n=25)	92	100
II (n=25)	90	82
III (n=16)		
Qualitative	97	94
Quantitative	75	69
IV (n=16)	72	64

Ultrasonography detected more follicles than present after slaughter (Table 16). Max et al. (1997) say this is due to a shift of the different levels or plains during ultrasonography when imaging the whole ovary.

Rectal palpation only detected 70% of all follicles. Follicles smaller than 10 mm were hard to detect with either method. For the detection of CLs, ultrasonography had worse results (85%) than rectal palpation (94%). Classification in groups based on ultrasonography and rectal palpation can be seen in Table 15. The quantitative classification of CLs in group III was moderate for both techniques, although the qualitative classification was good. For cows that have been superovulated ultrasonography was found to be a better method to examine the ovaries than rectal palpation, although it is not fully reliable.

Table 16. Percentages of correct diagnoses by the different methods, compared to inspection after slaughter (according to Max et al., 1997)

Ovarian structures (numbers and %)	Inspection after slaughter	Ultrasonography	Rectal palpation
CL	115 (100%)	98 (85%)	108 (94%)
Follicle	46 (100%)	50 (109%)	32 (70%)

2.6.8 Battocchio et al. (1999)

The accuracy of ultrasonography to detect and characterize CLs was determined by comparing the results with progesterone concentrations at the time of examination. In a first study with eight cows the ovaries were ultrasonographically examined and progesterone levels were measured daily from estrus to day 4, then at day 7, day 10, and again daily from day 17 to the onset of the next estrus. Attention was paid to the echogenic appearance of the CLs, and progesterone concentrations were used to classify the CLs functionally. In a second study, the ovaries of 157 Holstein Friesians were examined once by ultrasonography and a blood sample was collected and analyzed for progesterone. The examiner did not have any information about the stage of the estrous cycle a cow was in. Based on echogenic appearance, CLs were divided into three groups: absence of a CL, evolving CL, or mid-cycle CL. Based on progesterone levels, three additional groups were made in which the CLs were functionally classified: when progesterone concentrations were less than 1 ng/mL, no CL was present.

Table 17. Classification of CLs by ultrasonography and plasma progesterone concentration (n=157) (from Battocchio et al., 1999)

Ultrasonographic CL classification	Plasma progesterone concentration						
		less than 1ng/mL		1 to 4 ng/mL		more than 4 ng/mL	
	n	n	(%)	n	(%)	n	(%)
CL not detected	54	38	(70)***	16	(30)	0	0
Evolving CL	50	3	(6)	33	(67)***	14	(28)
Midcycle CL	53	0	0	6	(11)	47	(89)***

Note:*** Significant agreement between ultrasonography and progesterone values (P<0.001)

When concentrations were between 1 and 4 ng/mL, an evolving CL was present, and when progesterone concentrations exceeded 4 ng/mL, a CL was classified as being mid-cycle. The degree of agreement between the two techniques was calculated.

The first study produced the following results: CLs of days three and four were observed as dark grey structures with an indistinct border and a diameter of less than 20 mm. Progesterone concentrations during days three and four ranged from 1.1 to 1.7 ng/mL. A mid-cycle CL was a grayish well defined granular structure more than 20 mm in diameter with a demarcation line between it and the ovarian

Table 18. Ultrasonographic diagnosis of a functional CL compared to progesterone concentrations (n=157) (from Battocchio et al., 1999)

Ultrasonographic CL classification	Plasma progesterone concentration				
		less than 1ng/mL		more or equal to 1 ng/mL	
	n	n	(%)	n	(%)
CL not detected	54	38	(70)***	16	(30)
CL detected	103	3	(3)	100	(97)***

Note: *** Significant agreement between ultrasonography and progesterone values (P<0.001)

stroma. It could be observed between days 7 and 17 to 21 of the cycle and progesterone concentrations from 4.0 to 7.3 ng/mL were measured. On days 17 and 18 a CL could not be distinguished from those observed on days three and four of the estrous cycle, regardless of whether ultrasonography or progesterone were used. Developing and regressing CLs could thus not be discriminated and both were classified as evolving CLs.

The results of the second study are shown in Table 17. An overall high level of agreement existed between classification by ultrasonography and progesterone concentrations (kappa = 0.7206). A high level of agreement was also obtained when comparing ultrasonographic determination of the presence

Table 19. Ultrasonographic determination of the presence of a mid-cycle CLs compared to plasma progesterone concentration (n=157) (from Battocchio et al., 1999)

Ultrasonographic CL classification	Plasma progesterone concentration				
		less or equal to 4 ng/mL		more than 4 ng/mL	
	n	n	(%)	n	(%)
evolving CL	50	36	(72)***	14	(28)
midcycle CL	53	6	(11)	47	(89)***

Note: *** Significant agreement between ultrasonography and progesterone values (P<0.001)

of a CL with its functional status as assessed by progesterone concentrations of <1 ng/ml and ≥1 ng/ml (Table 18) (kappa = 0.7156). In Table 19 the diagnoses of the presence of mid-cycle CLs by both ultrasonography and progesterone concentrations were compared in animals with progesterone levels >4 ng/ml. The level of agreement between the two remained high (kappa = 0.6095).

Table 20. Comparison between classification based on measurements of diameter and progesterone concentrations (n=99) (from Veronesi et al., 2002)

Ultrasonographic CL classification	*n*	Plasma progesterone concentration					
		<1 ng/ml		1–4 ng/ml		>4 ng/ml	
		n	%	*n*	%	*n*	%
CL not detected	30	21	70***	9	30	0	0
CL Ø < 20 mm	22	4	18.2	13	59.1***	5	22.7
CL Ø ≥ 20 mm	47	2	4.2	7	14.9	38	80.9***

Note: *** Significant agreement between ultrasonographic measurements and progesterone values (P<0.001)

When classification of CLs was based on diameter alone (unpublished data), a low degree of agreement was found with progesterone concentration, particularly when the diameter was less than 20 mm.

2.6.9 Veronesi et al. (2002)

99 Friesian cows were examined by transrectal ultrasonography to assess the more reliable method for the functional classification of CLs: ultrasonographic measurement of the diameter of CLs, or ultrasonographic evaluation of the appearance of CLs. The measurement of the diameter was taken at least five times for each CL and only the greatest one was taken into account. The examiner had no information about the estrous cycle. These two data were compared with progesterone concentration from blood taken immediately before the ultrasound examination. The degree of agreement was then calculated. CLs were divided into different categories:

- Based on ultrasonographic measurement of the diameter, three groups were made: A) CL not detected, B) CL Ø<20mm, and C) CL Ø≥20mm.
- On the basis of ultrasonographic appearance, three groups were established: A) CL not detected, B) evolving CL, and C) mid-cycle CL.
- Based on progesterone concentration, three groups were established: A) CL not detected

Table 21. Classification based on ultrasonographic appearance compared to progesterone concentrations (n=99) (from Veronesi et al., 2002)

Ultrasonographic CL classification	*n*	Plasma progesterone concentration					
		<1 ng/ml		1–4 ng/ml		>4 ng/ml	
		n	%	*n*	%	*n*	%
CL not detected	30	21	70***	9	30	0	0
Evolving CL	25	5	20	16	64***	4	16
Mid-cycle CL	44	1	2.3	4	9.1	39	88.6***

Note: *** Significant agreement between ultrasonographic measurements and progesterone values (P<0.001)

when progesterone values were less than 1 ng/ml, B) evolving CL when concentrations were between 1 and 4 ng/ml inclusive, and C) mid-cycle CL when values were more than 4 ng/ml.

The category 'evolving' based on ultrasonographic appearance contained both developing and regressing CLs for the same reason as discussed for Battocchio et al. (1999). Also the same criteria as with Battocchio et al. (1999) are used to categorize CLs based on ultrasonographic appearance and progesterone concentration. Veronesi et al. (2002) observed some cystic CLs, but the size of the cavity was ignored in the measurements, since it is well known that no significant correlation exists between the presence or size of the cavity and progesterone concentrations.

Table 22. Ultrasonographic detection of CLs based on measurements of diameter compared with progesterone concentrations (n=99) (from Veronesi et al., 2002)

Ultrasonographic CL classification	*n*	Plasma progesterone concentration			
		<1 ng/ml		≥1 ng/ml	
		n	%	*n*	%
CL not detected	30	21	70***	9	30
CL detected	69	6	8.7	63	91.3***

Note: *** Significant agreement between ultrasonographic measurements and progesterone values (P<0.001)

An overall high level of agreement (kappa = 0.677) existed between classification based on ultrasonographic measurements and classification based on progesterone concentrations (Table 20). The same was true for ultrasonographic appearance (kappa = 0.732) (Table 21). A high level of agreement (kappa = 0.631) was also seen when comparing detection of CLs based on measurements of diameter, with progesterone concentration of <1 and ≥1 ng/ml (Table 22). Lower degree of agreement (kappa = 0.55 4) was found however, when the diagnosis of the mid-cycle CL presence by measurement was compared with the same diagnosis based on progesterone concentration (Table 23). Ultrasonographic detection of CLs based on appearance compared with progesterone concentrations of <1 and ≥1 ng/ml also showed high level of agreement (kappa = 0.631) (Table 24). This kappa value is the same as for early detection by measurement, as it is not possible to evaluate

Table 23. Diagnosis of mid-cycle CLs based on measurement compared with diagnosis of mid-cycle CLs based on progesterone concentrations (n=69) (from Veronesi et al., 2002)

Ultrasonographic CL classification	*n*	Plasma progesterone concentration			
		≤4 ng/ml		>4 ng/ml	
		n	%	*n*	%
CL Ø < 20 mm	22	17	77.3***	5	22.7
CL Ø ≥ 20 mm	47	9	19.1	38	80.9***

Note: *** Significant agreement between ultrasonographic measurements and progesterone values (P<0.001)

Table 24. Ultrasonographic detection of CLs bases on appearance compared with progesterone concentrations (n=99) (from Veronesi et al., 2002)

Ultrasonographic CL classification	*n*	Plasma progesterone concentration			
		<1 ng/ml		≥1 ng/ml	
		n	%	*n*	%
CL not detected	30	21	70***	9	30
CL detected	69	6	8.7	63	91.3***

Note: *** Significant agreement between ultrasonographic measurements and progesterone values (P<0.001)

appearance and diameter of a CL if it is not detected by ultrasonography at all. Finally, diagnosis of mid-cycle CLs based on appearance was compared with progesterone concentrations (kappa = 0.720) (Table 25). This last kappa value shows that ultrasonographic appearance is a better method for diagnosing mid-cycle CLs than measuring them. Seven false positive diagnoses were made for the presence of a CL, independent of the type of the method of classification (appearance or size). This was probably due to misinterpretation of the ultrasonic image by the examiner, but might also be the results of the golden standard not being completely right.

Table 25. Ultrasonographic classification of CLs based on appearance compared with plasma progesterone concentrations (from Veronesi et al., 2002)

Ultrasonographic CL classification	*n*	Plasma progesterone concentration			
		≤4 ng/ml		>4 ng/ml	
		n	%	*n*	%
Evolving CL	25	21	84***	4	16
Mid-cycle CL	44	5	11.4	39	88.6***

Note: *** Significant agreement between ultrasonographic appearance and progesterone values (P<0.001)

2.6.10 Conclusion of research by others

In order to perform a well designed study, the cycle stage needs to be determined. This can be done by measuring progesterone concentrations or after synchronizing the cows. Many studies have been performed to determine the cycle stage in cows by rectal palpation and ultrasonography. Both seem to be good methods for detecting mid-cycle CLs. Evolving CLs are, however, more difficult to detect. Non of the cited studies examined the accuracy of the combination of rectal palpation with ultrasonography.

3. RESEARCH

3.1 Material and methods

In the slaughter house, 49 dairy cows with unknown reproductive history have been examined with the anamnesis of 'not seen in estrus'. We chose for this anamnesis because the differential diagnosis contains all thinkable features going from inactive ovaries to pregnancy. The purpose of the study was to 1) set the diagnosis and determine the accuracy of rectal palpation and rectal ultrasonography herein and 2) determine the cycle stage in cycling animals.

During the first part of the study, the ovaries of 23 cows have been examined. First a rectal palpation was performed, immediately followed by transrectal ultrasonography. To perform ultrasonography, the Tringa® Linear Vet ultrasound machine (Easote) equipped with a linear multifrequency (5-7.5 MHz) probe was used. To visualize the ovarian structures, both the 5 and 7.5 scan frequency were used. Both examinations were carried out by the same experienced investigator 12-20 hours before slaughter. After slaughter, the ovaries were dissected by another investigator.

When the diagnosis 'subestrus' was made, the ovaries would be classified into three categories: Stage 1 from d1-4, stage 2 from d5-16 and stage 3 from d17-21 (cfr Pieterse et al., 1990). Stage 2 corresponds with the days during which a CL would be sensitive to a treatment with PG. The ovaries were classified mainly based on the size, firmness and aspect of the CL (cfr Pieterse et al., 1990). As mentioned above, only the findings on the largest ovary were taken into account to identify the stage of the estrous cycle. No attention was paid to the uterus.

- Stage 1
    - Palpation: The CL is soft, small, and lies on the surface of the ovary. Follicles can be present.
    - Ultrasonography: The CL appears as a small, poorly defined, irregular, greyish-black structure with echogenic spots and lies all within the contour of the ovary. Follicles can be present.
- Stage 2
    - Palpation: The CL has a liver-like consistency, is more defined, and sometimes protrudes above the surface of the ovary. Follicles can be present.
    - Ultrasonography: The CL is a well defined granular, greyish, echogenic structure. A demarcation line is visible between the CL and the ovarian stroma. Follicles can be present.
- Stage 3
    - Palpation: The CL is less protruding than in stage 2, more compact, and almost as firm as the ovarian stroma. ≥1 Large, firm follicle is present.
    - Ultrasonography: The CL is small and the demarcation line is faint. ≥1 Large follicle is present.

Based on the morphological findings after dissection the ovaries of cycling cows were again classified into three categories. These categories are based on the four stages of Ireland et al. (1980), from which stage 2 and 3 have been combined into one single stage in order to have categories similar to those listed above: Stage 1 from d1-4, stage 2 from d5-17 and stage 3 from d18-21 (Ireland et al., 1980):

- Stage 1: Point of rupture is not covered by epithelium, a CH is present/ the apex of a CL is forming, small follicles can be present (see Figure 8).
- Stage 2:
    - D5-10: The ovary contains a fully formed CL with vasculature visible around its periphery. When bisected, the apex is red or brown, while the remainder is orange or yellow. Follicles can be present.
    - D11-17: The entire CL is bright orange or yellow. Late in this stage vasculature will be visible over the apex of the CL. Follicles may be present.
- Stage 3: ≥1 Large follicle (10 mm or more) and a regressed CL, with no vasculature visible on its surface, are present. Externally, the CL is light yellow to white.

When cows were diagnosed as being non-cyclic, they could, of course, not take part in this study. The diagnosis of a non-cyclic cow was made by rectal palpation and ultrasonography, and confirmed after slaughter. The definitions for non-cycling cows are:

- Cysts: Follicle-like structures bigger than 2.5 cm, either with a thin wall (<3 mm; follicular cyst) or with a thick wall (>3 mm; luteal cyst). When cysts are present, there will be no CL and the uterus will not be contractile.
- Puerperium: The first 40 days following parturition, during which uterine involution takes place.
- Pyometra: A chronic endometritis with a persistent CL. Cows will have a (muco)purulent discharge, a cervical diameter larger than 7.5 cm and asymmetry of the uterine horns.
- Pregnancy: The condition of having a developing embryo or fetus in the uterus.
- True anestrous: The condition during which the ovaries are not active. Both ovaries will be small and hard. There may be a small follicle present, but never a CL.

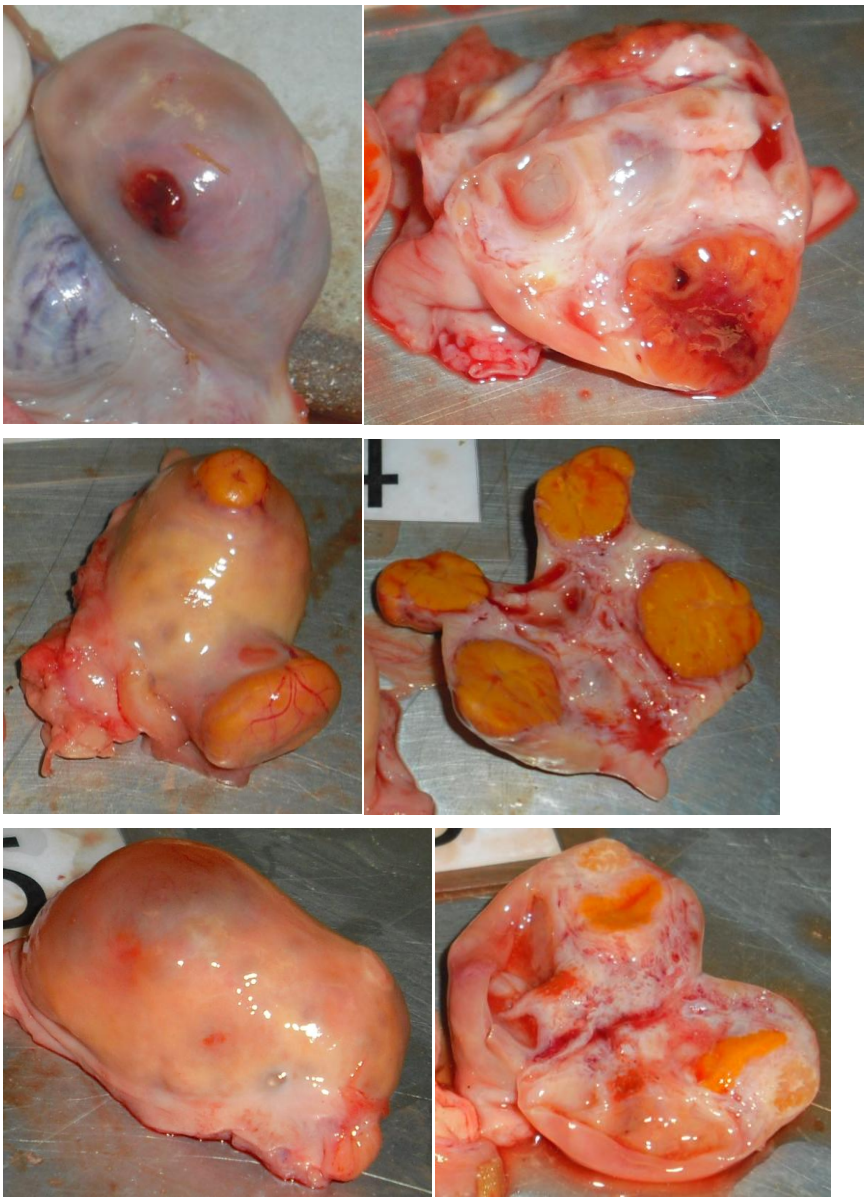

Figure 8. Both intact and dissected ovaria of resp. stages 1 (a CH), 2 (2 CLs) and 3 (a CL and a large follicle)

3.2 Results

From the 23 cows in the first part of the study, two could not be classified because of doubt about the diagnosis:

- For one cow the investigator was in doubt during rectal examination whether the cow had just ovulated or if it was in anestrus. At inspection after slaughter a CH was found.
- The other cow was, based on rectal examination, classified as being in stage 2 although the CL was not protruding. Its consistency was, however, liver-like and on ultrasound a clear demarcation line could be seen between the CL and the ovarian stroma. At inspection after slaughter there was doubt between stage 1 and stage 3. The left ovary was inactive and the right ovary contained an old CL, a CH and 1 follicle (see Figure 9).

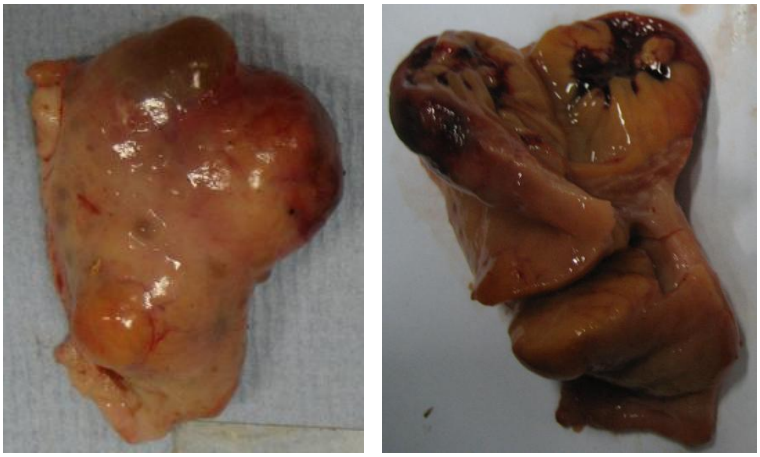

Figure 9. Ovary with CH, CL and follicle

The results from the remaining 21 cows can be seen in Table 26. Thirteen cows were found to be cycling, of which 11 had been diagnosed correctly. The two misinterpretations are both between stages 1 and 3. All cows with cysts, anestrus, and pregnancy were correctly diagnosed. Remarkably there was one cow in which a CH was found in the presence of a cyst-like structure. According to the definition of a cyst, this cyst-like structure has no pathological role and is hence named 'indifferent cyst'.

For the second part of the experiment, 26 cows with unknown reproductive history have been examined. The methods used were similar to the first part of the experiment, with the exception of the order in which rectal palpation and ultrasonography took place: the ovaries were first palpated for the detection of CLs and other structures. Then ultrasonography was performed to identify the stage of estrous cycle based on echogenicity and aspect. Finally, rectal palpation was used to reinforce the diagnosis. Also the definitions from Pieterse et al. (1990) and Ireland et al. (1980) were not applied as strictly as before, as there was some doubt if they were 100% correct. Slaughter now took place 0.5 to

Table 26. Results from the first part of the study (n=21)

		Inspection after slaughter							
		Stage 1	Stage 2	Stage 3	Cyst	Anestrus	Pregnant	Pyometra	Puerperium
Rectal palpation and ultrasonography	Stage 1			1					
	Stage 2		8						
	Stage 3	1		3					
	Cyst	1			2				
	Anestrus					1			
	Pregnant						3		
	Pyometra								
	Puerperium		1						

3 hours after examination.

From the 26 cows, two could not be classified because of doubt about the diagnosis:

- One cow was diagnosed as stage 1 during rectal examination, but at inspection after slaughter there was doubt between stage 1 and stage 2. Although the CL was entirely bright orange and

vasculature was visible over its apex, it was not protruding, was soft and small blood cloths were present inside of it.

- For another cow, rectal examination could not distinguish between anestrus and puerperium.

From the remaining 24 cows, the results can be seen in Table 27. As before, all non-cycling cows (n=15) were correctly diagnosed by rectal palpation and ultrasound. In one of the cows with the cyst, there was also a protruding CL present (Figure 10). Again, this is in contradiction to the definition of a cyst (see above). The other cow with the cyst also had a CL, but this CL was older (Figure 11).

Table 27. Results of second part of the study (n=24)

		Inspection after slaughter							
		Stage 1	Stage 2	Stage 3	Cyst	Anestrus	Pregnant	Pyometra	Puerperium
Rectal palpation and ultrasonography	Stage 1		2	1					
	Stage 2		3						
	Stage 3		1	2					
	Cyst				2				
	Anestrus					1			
	Pregnant						11		
	Pyometra								
	Puerperium								1

Because the total number of cycling cows was very low in this study, calculating sensitivity, specificity, positive and negative predictive value would not be of great value. In general it can be said though, that rectal palpation and transrectal ultrasonography are good methods to diagnose non-cyclic cows. For cyclic cows, however, there are too few clear rules for rectal palpation, ultrasonography, and inspection of the ovaries after slaughter. Most authors, for example, mention the existence of a cystic CL, but none of them include this structure in their table to define the cyclic stage. More research needs to be performed, preferably when it is known which stage of the cycle the cows are in.

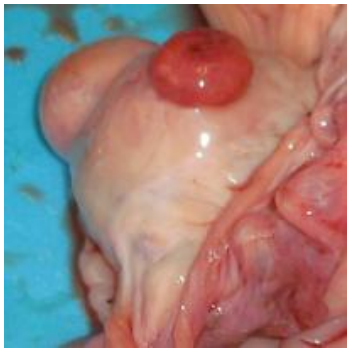

Figure 10. Protruding CL in the presence of a cyst (left)

3.3 Discussion

Partially due to the high number of pregnant cows, not many conclusions can be drawn from this study. However, some difficulties were noticed during both the rectal examination and the inspection of the ovaries after slaughter. A similar, but larger study needs to be carried out in order to conclusively prove the classifications made by Pieterse et al. (1990) and Ireland et al. (1980) are correct. There are currently some lingering doubts (discussed below). It would have been better to include progesterone concentrations in this study as

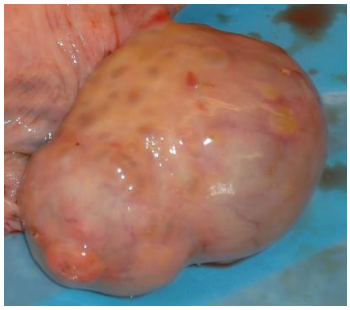

Figure 11. Older CL (left) in the presence of a cyst

inspection of the ovaries after slaughter alone is not a good golden standard. There was however, insufficient funding available to do this.

One of the reasons why it is thought after this study that the classifications from Pieterse et al. (1990) and Ireland et al. (1980) are not completely correct is this: in the second part of this study, both cows that were classified in stage 1 after rectal palpation and ultrasonography and in stage 2 after slaughter, had a soft CL although being entirely bright orange and having vasculature visible over the apex. These findings did not conform to the classifications that were used. According to Pieterse et al. (1990) a CL from stage 2 should be of liver-like consistency and not soft. It would be good to re-evaluate these classifications with known cycle-stages in all cows.

Only very limited or no visible differences can be seen in the echogenicity of CLs during the different stages of the estrous cycle (Grygar et al., 2002). Just like with Battocchio et al. (1999), Hanzen et al. (2000), Pieterse et al. (1990) and Ribadu et al. (1994) some difficulties were noticed when attempting to discriminate between developing and regressing CLs. The latter only identified 1 of the 3 regressing CLs observed postmortem by ultrasonography. This may be caused by the lack of echogenic differences between the regressing CL and the ovarian stroma. Rectal palpation could be valuable in these cases of doubt since the firmness of the CL can be of great help. Battocchio et al. (1999) even mention that the results of their study confirm that ultrasonography alone is not sufficient to determine the age of a CL.

For the detection of mid-cycle CLs with ultrasonography, different authors have found a high accuracy (Pieterse et al., 1990; Ribadu et al., 1994). Although no accuracy was calculated in this study, it can be said that mid-cycle CLs were detected better than CLs from stage 1 or 2.

Even though Pieterse et al. (1990) use transvaginal ultrasonography in their study, the transrectal method is more commonly used. The latter has also proven to have higher accuracy (Grygar et al., 1992). Pieterse et al. (1990) could not detect eight CLs with transvaginal ultrasonography compared to 57 found on dissection. On the other hand, Pierson and Ginther (1987) found a 100% agreement between transrectal ultrasonography and dissection to detect CLs. The eight CLs that were missed by Pieterse et al. (1990) were all in stage 1 or stage 3 of the cycle. Besides the different method of ultrasonography, other factors (such as bad quality of the ultrasound) could also have contributed to these misdiagnoses.

When looking at rectal palpation, it is best to make this the first step of the examination. Grygar et al. (1992) chose to start with ultrasonography in half of their cows, but later mentioned the considerable disadvantage of this order.

With some studies, classification of CLs was based on diameter alone. A low correlation was found with progesterone concentration by Battocchio et al. (1999), particularly when the diameter was less than 20 mm. In contrast, Ribadu at al. (1994) found a high degree of agreement between CL diameters and plasma P4, but they observed most of the discrepancies on days 18-21, during the CL

involution. Diameters seemed to decrease more slowly than P4 concentration in this last phase of the estrus cycle.

It is generally known that progesterone concentrations higher than 1 ng/ml indicate the presence of a functional CL. There is still disagreement though, about progesterone concentrations indicating the presence of a fully active or mid-cycle CL. Different authors have been using different concentrations. Battocchio et al. (1999) have used 4 ng/ml, as during their study plasma progesterone never fell below this level during diestrus.

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