Analysis of ovaries in studies of reproduction in red deer (*Cervus elaphus*, L.): Application and limitations

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Abstract: A set of definitions for luteal structures and their regressing stages in red deer ovaries is suggested. Structural characteristics in ovaries pertaining to reproductive analysis is compiled from relevant literature and combined with observations from the present study. Luteal structures and their regressing stages may be useful in assessment of reproductive status and history, provided the analysis is performed with a full understanding of the limitations of the criteria and the methodological approach. Primary *corpus luteum* (PCL), corpus luteum of pregnancy (CLV), and *corpus rubrum* (CR) are the most important structures in the quantitative analysis of reproduction, and they may be identified at a macroscopic level. However, confusion with other structures is conceiveable, and for an accurate analysis microscopic examination of histological preparations is necessary. Different processing and analysing procedures are compared, illustrating differences in resolution and precision, especially in retrospective analysis. Data from hinds with known reproductive history indicate limitations and potential in analysis of ovaries as a technique to assess reproductive status and history in red deer.

Key words: Red deer, ovaries, analysis, luteal structures, definitions, histology, development, methods, accuracy.

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Introduction

Since the work of Cheatum and Morton (1942, 1946) and Cheatum (1949), the analysis of ovaries has been widely applied to quantify reproductive parameters in wild cervids (*Odocoileus:* Golley 1957; Mansell 1974; Thomas 1983, *Capreolus:* Strandgaard 1972; Horak 1989, *Rangifer:* Dauphiné and McClure 1974; Leader-Williams and Rosser 1983, *Alces:* Markgren 1969; Sæther and Haagenrud 1985, *Cervus:* Mitchell 1973; Wegge 1975). Large mammals exhibit great differences in ovarian histology (Harrison 1948; Uyttenbroeck and van der Scheueren-Lodeweyekx 1969; Harrison and Weir 1977), and significant interspecific variation in structural dynamics in the ovaries during reproductive development and cycles has been documented (Valentincic 1958; Buss and Smith 1966; Markgren 1969; Mansell 1971; Harrison and Weir 1977; Leader-Williams and Rosser 1983; Lockyer 1987; Horak 1989). Comprehensive literature reviews by Zuckerman and Weir (1977) and Jones (1978) emphasize that interspecific differences in ovarian histology need to be considered in clinical and analytical work, and there is still a requirement for more detailed reference descriptions in various species.

In histological analysis of ovaries, luteal structures and their various regressing stages are the main source of information for determining parameters such as estrus period, ovulation rates, and previous breeding events, but few reports (e.g. Morrison 1960), describe ovarian structures in relation to animals of known age, reproductive status and breeding history. Different definitions and descriptions of histological structures have been applied in the analysis (Morrison 1960; Buss and Smith 1966; Markgren 1969; Mansell 1971; Harrison and Weir 1977; Harder and Moorhead 1980; Thomas 1983) and methodological problems may bias the interpretation of origin, characteristics, and persistence of various structures (Halazon and Buechner 1956; Golley 1957; Buss and Smith 1966; Mansell 1971; Thomas 1983). For example comparing results from different studies may sometimes be difficult since different preservation methods (freezing and/or chemical preservation), processing protocols and analytical techniques (proper histological preparation of microscopic slides or macroscopic inspection of handsliced, non-dyed ovaries) have been applied (Valentinicic 1958; Morrison 1960; Trauger and Haugen 1965; Mansell 1971; Brokx 1971; Wegge 1975). Different processing and analytical procedures are likely to produce different limitations in the success of retrospectively identifying previous parturitions and regressing luteal structures.

The current literature seems somewhat inconsistent regarding terminology and definitions of luteal structures and their regressing stages, and descriptions of various structures appear scattered and insufficient, especially at the species level. Therefore, this paper suggests a set of definitions for ovarian structures pertaining to analysis of ovaries for management purposes. The characteristics of various structures are described from a compilation of relevant literature, and related to actual reproductive events in known-aged female red deer. Morphometric descriptions and occurrence of ovarian structures are presented throughout the annual cycle. Also, analysis of ovaries from females with known reproductive status and history is summarized to illustrate the limitations of applied histological criteria, processing procedures, and level of analysis.

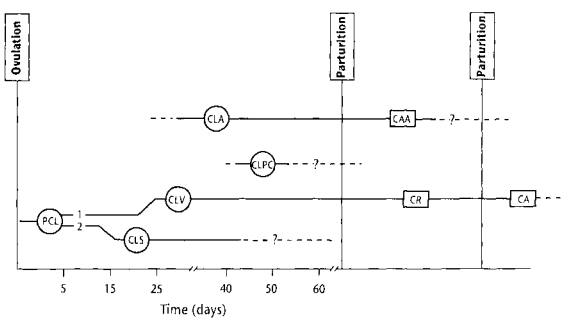

Fig. 1. Visual presentation of definitions of luteal structures and their regressing stages in relation to significant events in reproduction. 1=in case of conception, 2=no conception.

Material and methods

Definitions and characteristics of ovarian structures.

In the literature describing various ovarian structures in mammals, inconsistency in the terminology applies especially to the post partum *corups albicans* and different stages of the *corpus luteum* (Halazon and Buechner 1956; Valentinic 1958; Morrison 1960; Buss and Smith 1966; Mansell 1971; McDonald 1975; Dellmann and Brown 1976). Also, various types of luteal structures and their regressed stages are not always distinguished for a precise association with specific reproductive events. As a result, problems in estimating reproductive parameters may arise (Cheatum 1949; Golley 1957; Morrison 1960; Trauger and Haugen 1965; Mansell 1971; Brodie 1972; Brokx 1972).

Although morphometry and histological structures in cervid ovaries are reported from many studies (e.g. Cheatum 1949; Valentincic 1958; Morrison 1960; Markgren 1969; Leader-Williams and Rosser 1983; Horak 1989), the information has not yet been systematically put together for the different species, and few authors have presented descriptions and definitions of various structures suitable for methodological application (Morrison 1960; Mansell 1971, 1974; Thomas 1983). Primarily, the definitions below are relevant for reproductive studies in seasonal breeders usually giving birth to a single offspring (e.g. red deer and reindeer). Structural characteristics pertaining to red deer ovaries are compiled on the basis of existing literature. The following ovarian structures (Fig. 1) are distinguished:

1. Primary corpus luteum (PCL)

Definition: Luteal structure present during metestrus and towards the end of diestrus, – and

in the case of pregnancy until a conceptus can be verified. PCL develops from an ovulated Graafian follicle, it is not associated with a conceptus, and by definition it can turn into a CLS *(corpus luteum spurium)* or a CLV (*corpus luteum verum*, - see McDonald 1975, Fig. 1, and below), depending on the progress of the reproduction cycle (non-conception or conception).

Characteristics: Mean diameter of the established PCL 4-10 mm (Valentincic 1958; Kelly and Challies 1978), increasing in size at least until the ruptured follicle is fully luteinized after 6-9 days (Harrison 1948; Morrison 1960; McDonald 1975; Nalbandov 1976; Harrison and Weir 1977). Fully luteinized PCL are generally round or oblong with regular outline, usually positioned close to the tunica of the ovary. The colour after fixation varies from pale yellow to yellow-grey (Morrison 1960; Trauger and Haugen 1965). PCL are sometimes infiltrated with stringes of haemorrhagic exudate (Harrison and Weir 1977). Recently ruptured follicles often have a layer of luteal cells of varying thickness lining the folded follicular wall, leaving a central cavity with antral fluid sometimes mixed with blood (*corpus hamorrhagicum*, - Dellmann and Brown 1976; Harrison and Weir 1977). The associated ovulation aperture is healed after 2-4 days (Harrison 1948), but is still obvious, often surrounded by a network of blood vessels, and sometimes extruding on the surface like a ring-formed ridge (Morrison 1960).

At the cellular level PCL in early stages (<4 days) contain granulosa cells in transitional stages as well as developed, oval granulosa lutein cells with a average diameter of ca. 25 microns (Valentincic 1958). By the fifth to sixth day granulosa cells are fully luteinized (Harrison and Weir 1977), infiltrated by capillaries from the surrounding theca interna, and lipid-containing teca lutein cells have dispersed among granulosa lutein cells with associated disruption of the membrana propria (Harrison 1948; McDonald 1975; see also Mossman and Duke 1973). Theca lutein cells, being the smaller type of luteal cells, make up for a minor part of the luteal tissue, and occupy mainly trabecular and peripheral areas of the PCL (Dellmann and Brown 1976). Growth in the PCL caused by hypertrophy and probably hyperplasia in luteal cells levels off in mid-diestrus of the cycle (Harrison 1948; McDonald 1975; Nalbandov 1976). The PCL is by then fully vascularized with enlarged thecal blood capillaries and smaller vessels separating columns of luteal cells (Harrison 1948; Valentincic 1958; McDonald 1976; Dellmann and Brown 1976).

2. Corpus luteum spurium (CLS, - McDonald 1975)

Definition: Regressing luteal structure derived from a PCL during late diestrus as a result of failed conception. CLS is functionally not connected to a conceptus, but may occur in association with an implant when the conception has taken place during an estrous cycle subsequent to the one leading to the CLS. By definition, CLS is confined to the season of estrous cycles and pregnancy.

Characteristics: Mean diameter 2-8 mm (Halazon and Buechner 1956; Morrison 1960; Kelly and Challies 1978), decreasing rapidly from late diestrus. CLS is probably hard to identify after 2-3 succeeding cycles or at least after the first trimester of pregnancy (Harrison 1948; Morrison 1960; McDonald 1975; Dellmann and Brown 1976; Nalbandov 1976). The superficial ovulation rupture associated with CLS is always healed and non-haemorrhagic, but usually easy to identify as a small surface depression.

Regression in CLS starts 12-15 days post ovulation (Harrison 1948; Dellmann and Brown 1976; Nalbandov 1976). Progesterone production plummets at the same time, even more dramatically than the associated anatomical changes (McDonald 1975). Involution of the CLS begins with degenerative changes in blood vessels, including increased pycnosis of endothelial cell nuclei.

Capillaries are mostly confined to the periphery of the corpus (Dauphiné and McClure 1974). Also, there is a marked peripheral vacuolation and shrinkage of luteal cells which become more irregular in size and shape with diffuse contours (Valentincic 1958; Harrison and Weir 1977). The luteal cell cytoplasma contains gradually more granular bodies and the eccentric nuclei are fragmentated. Intercellular space is irregular and tends to increase (Valentincic op.cit.). As luteal regression continues, there is an increased condensation of lutein pigment, and the CLS may take on a gradually more orange or reddish-brown colour. However, as necrotic luteal cells eventually are replaced by

fibroblasts, and other cells become enmeshed in the forming connective tissue, the amount of lipochrome pigment decreases and the CLS appear inconspicuously greyish-white (Morrison 1960; McDonald 1975; Harrison and Weir 1977).

CLS shows rapidly increasing convolution and wrinkling of its outline and thecal layers, trabecular strands of connective tissue infiltrate the luteal body, and hyaline material may occur (Nalbandov 1976). After 3 to 4 weeks CLS appears as an irregular structure with its outline disintegrated and consisting mainly of vascular elements and connective tissue (Golley 1957). The remaining luteal cells are highly necrotic, disorganized and occur as more or less pigmented elements scattered in an increasing network of fibroblasts and connective tissue (Harrison 1948; Valentincic 1958; Morrison 1960; Harrison and Weir 1977).

Trauger and Haugen (1965) found that textural characteristics were helpful for differentiating CLS and CLV in white-tailed deer, the former structure having a rougher texture and a harder surface.

3. Corpus luteum verum (CLV, – McDonald 1975)

Definition: Luteal structure originating from an ovulated Graafian follicle and functionally related to an implant.

Characteristics: Mean diameter 7–16 mm (Halazon and Buechner 1956; Valentincic 1958; Morrison 1960; Kelly and Challies 1978). The diameter of the developed CLV relates to the diameter of the follicle from which it arose, but there is variation from near equality to a two-fold increase over that of the follicle (Harrison 1948; Harrison and Weir 1977). CLV tends to increase in size at least until the 70th day of gestation (Morrison 1960). Superficial ovulation rupture is healed and non-haemorrhagic, but is often visible, particularly through the first trimester of pregnancy.

Except for the generally larger size, the CLV is morphologically and histologically rather similar to the developed PCL (Morrison 1960). Valentincic (1958) describes luteal cells in the CLV as rounded, oblong with somewhat pointed ends, and with a mean diameter of large lutein cells (granulosa origin) approximating 35 microns. This is somewhat larger than the corresponding cells of the PCL. The cell membranes in the CLV are more distinct than in the PCL, and the cytoplasma is rather homogenous or finely granulated around the large transparent nuclei (Valentincic op. cit.; Dauphine and McClure 1974). The CLV may contain a relatively smaller population of theca lutein cells compared to PCL. In some artiodactyles a possible distinction between CLV and PCL can be demonstrated by differences in phosphorylase activity, which is observed in lutein cells in CLV, but confined to vascular walls in PCL (Harrison and Weir 1977). Differences in size of the luteal cells between PCL and CLV have also been reported in caribou, where in addition capillaries of PCL seem confined to the periphery of corpus, and luteal cells show considerable intercellular space (Dauphiné and McClure 1974).

4. Corpus luteum accessorium (CLA)

Definition: Luteinized, non-ruptured follicle present in the same pair of ovaries as one or more of the other luteal structures defined.

Characteristics: Mean diameter 2–5 mm (Morrison 1960; Kelly and Challies 1978). The CLA occurs as densely luteinized bodies as well as follicles with a layer of luteal cells lining the often irregular follicular wall, leaving an antrum with liquor folliculi (Weir and Rowlands 1977). Small luteinized bodies are often peripheral in the ovarian cortex with thecal layers poorly developed. Larger bodies tend to occur deeper and often with a hypertrophied thecal layer. Luteinizing atretic follicles sometimes appear collapsed with an irregular antrum and folded thecal layer. The basement membrane may be folded and increased in thickness (Byskov 1978). Apart from their generally smaller size, (Morrison 1960; Douglas 1966), and the presence of an entrapped oocyt or ovum, often necrotic, the CLA can be difficult to distinguish from a true PCL or CLV (Weir and Rowlands 1977). By definition, CLA comprise all types of luteal bodies not derived from an ovulated follicle.

5. Corpus luteum of post conception (CLPC, – Halazon and Buechner 1956)

Definition: Luteal structure related to a recent ovulation rupture occurring in the same pair of

ovaries as a CLV. The CLPC derives from a mature follicle ruptured after the one that developed into a CLV.

Characteristics: Mean average diameter 5–10 mm. Histological appearance similar to the developed PCL and CLV. Ovulation scar visible, appearing more recent than scars associated with the PCL or CLV (Halazon and Buechner 1956; Morrison 1960).

6. Corpus rubrum (CR, – Markgren 1969; Halazon and Buechner 1956; Morrison 1960)

Definition: Regressing luteal structure less than approximately 12 months old (post partum) derived from a CLV of the immediate preceding gestation.

Characteristics: The ovulation scar associated with a CR is always healed, but often visible, especially when the CR is located close to the ovarian surface. The CR decreases rapidly in size after parturition. Young CR (1–4 weeks) have a mean diameter of 3–6 mm and are mostly round or elongated in shape with a fairly regular outline containing an increasing amount of hyaline material and connective tissue (Morrison 1960; Dauphiné and McClure 1974; Mansell 1971; Harrison and Weir 1977; Leader-Williams and Rosser 1983). Older CR are smaller (1–3 mm), more diffuse in contour, contain more connective tissue, and are often distorted or flattened due to growing follicles or new corpora lutea (Halazon and Buechner 1956; Morrison 1960; Markgren 1969).

Degenerating capillaries with collapsed lumens and pycnotic endothelial cell nuclei are one of the early characteristics in CR regression. As CR increase in age, regressing luteal cells become fewer and increasingly more vacuolated and fragmented. Disintegration of luteal cells follows the same pattern as in CLS with vacuolation, granulation of cytoplasma and pycnosis of cell nuclei (Valentincic 1958; Dauphiné and McClure 1974; McDonald 1975). Up to an age of at least 4–5 months the great majority of CR are conspicuously pigmented, – bright orange or reddish brown (Golley 1957; Morrison 1960; Mansell 1974). At later regression stages the pigmentation usually becomes more diffuse, pale brown and less continuous. However, some CR turn distinctly brown without the tint of orange or red, while others show almost no pigmentation after 7–8 months (see Morrison 1960 for a detailed description).

Seven to eight weeks post partum the CR may still contain numerous necrotic luteal cells (Golley 1957) scattered among strands of hyaline material and connective tissue, resembling the histological picture of early regression stages in CLS. Pigmentation in young CR appears to be associated with degenerating luteal cells, while in older CR, with fewer luteal cells, pigment crystals (probably hemosiderin granula, – Golley 1957) are scattered among connective tissue cells (Morrison 1960). The rich vascularization of the CLV gradually disappears in the regression process occurring after parturition, although numerous groups of blood vessels and clumps of smaller, often peripheral, vascular elements scattered between strands of connective tissue characterize the CR.

7. Corpus albicans (CA)

Definition: Regressed luteal structure derived from a CLV related to a gestation terminated more than approximately 12 months ago. Hence, CA represents further regression stages of CR.

Characteristics: Size small (<2 mm – Morrison 1960; Dauphiné and McClure 1974), but generally difficult to determine due to irregular and often scattered scar tissue (Morrison 1960; Markgren 1969). The associated ovulation scar is inconspicuous and frequently difficult to identify. Discontinuous pigmented areas may occur (grey orange to pale brown or dark brown), but CA most often appear unpigmented and may be hard to identify macroscopically (Morrison 1960).

At the microscopic level CA are identified by groups of thick-walled blood vessels, scattered in areas of connective tissue. Finer vascular elements are rare or absent. If remnants of lutein cells occurr, they are highly necrotic and confined to clumps of unidentifiable cell debris. Hyaline material is scarse or usually absent (Mansell 1971; Dauphiné and McClure 1974). Pigment granules are seen both within degenerated luteal cells and wedged between connective tissue cells (Morrison 1960; Mansell 1971). The oldest identifiable CA consist of a few thick-walled blood vessels scattered among con-

nective tissue fibers confined to a matrix which gradually resembles the ovarian stroma (Mansell 1971).

8. Corpus albicans accessorium (CAA)

Definition: Regressing luteal structure less than approximately 12 months old, derived from CLA, and present in the same pair of ovaries as a CR.

Characteristics: Mean diameter <3 mm, shape and outline is fairly regular, round or oblong (Morrison 1960). Pigmentation is similar or less conspicuous compared to CR (Morrison 1960). Histologically CAA differ from CA, the latter representing more regressed stages of luteal structures. Except for smaller size, CAA resembles CR, especially its earlier stages (<2 months). Necrotic luteal cells are common between trabecular strands of connective tissue and hyaline material (Golley 1957). Vascular elements are less conspicuous than in CR, and mostly confined to periphery (see Dauphiné and McClure 1974).

For a possible survey and convenience, some characteristics of the structures defined are summarized in Table 1. In addition to literature referred to above, observations from this study are also included in the description. However, Table 1 is only partly satisfactory and sufficiently precise for an accurate identification of the structures. It should be emphazized that the definitions chosen in this study (Fig. 1) are designed for practical application of ovarian analysis as a way of revealing reproductive events retrospectively, as well as assessing of present status. Consequently, they do not necessarily meet the required precision within the framework of detailed histological descriptions and functional definitions.

The occurrence of corpora lutea structures is limited to the period of recurring estrous cycles and pregnancy. In a polyestrous, seasonal breeder like red deer that means in practice the period September to June. CLS is defined as a luteal structure, even though it represents stages in corpus albicans formation (McDonald 1975; Nalbandov 1976). However, the CLS is structurally and functionally completed within one estrous cycle (histologically manifest in late diestrus or proestrus). In its preceding stage (by definition PCL) the CLS may in some species play an important endocrinological role through its progestogenic activity during diestrus of the same cycle. This includes priming of the reproductive system for the succeeding cycle (behaviourally as well as physiologically) in species where "silent heat" is a frequent phenomena (McDonald 1975; Harrison and Weir 1977).

From the above it follows that various types and stages of corpora albicantia (CR, CA, CAA) arise from luteal structures developed during or prior to the last gestation. In cases where a female does not conceive after the last estrous cycle in a season (pathway diestrus-anestrus), regressing luteal structures from that season are said to belong to the "albicantia-group" only after a set date for the normal period of parturition the following spring. In this study as well as in the work by Clutton-Brock *et al.* (1982) June 1 is chosen as a suitable date for red deer.

Sampling and processing procedures.

Material

Ovaries from four groups of hinds were available for this study:

1. Eight hinds reared under near-natural conditions in an enclousre (20 ha) at Songli Research Station, Orkdal, Norway, were subject to controlled breeding regimes throughout their lives (Tab. 4). Estrous behaviour was closely observed from September to December, and subcutaneous and intraabdominal telemetry tags (Mohus 1976) provided information on temperature development during estrous cycles, thus indicating time of ovulation. For the same purpose, a modified version of a device for measuring conductance in vaginal mucus (Refsdal 1974; Kiddy 1979) was used 3–6 days around expected estrus. When forthcoming estrus was indicated, the hinds were exposed to mature stags for up to 5 days or until mating was confirmed. The hinds were slaughtered at different stages of the estrous cycle and in early pregnancy in order to study appearance and persistence of ovarian structures relating to specific reproductive events.

2. From a marked population of freeranging red deer, ovaries were available from 35 hinds aged 2–11 years. These females were immobilized and marked during winter as calves or yearlings. In subsequent winters they were re-captured and checked for signs of lactation during the previous summer and autumn (residual glandular tissue in udder). Observations were also made to assess if they had a calf associating with them. Thus with time, substantial information on reproductive history for specific hinds was accumulated. When the hinds were shot during subsequent hunting seasons, or died from other causes, uteri with ovaries along with the lower jawbone were handed in by hunters or local wildlife boards (see below). Analysis of ovaries from these marked hinds was aimed specifically at relatiang luteal scars to known number of parturitions, and to compare processing and analysing procedures.
3. Between 1970–1990 jawbones and reproductive tracts from 2554 hinds were collected in cooperation with hunters and local wildlife boards during the hunting season (Sept.–Nov.). In this material, 1655 specimens were complete with uterus and intact ovaries containing luteal structures and/or their regressing stages. The hunters were also asked to record date, locality, dressed weight, state of lactation and whether a calf was associated with the hind (Langvatn and Albon 1986). This material provided an opportunity to characterize ovarian structures during a period of high reproductive activity.
4. Outside the hunting season (Dec.–Aug.), reproductive tracts and jawbones from 110 hinds killed in accidents as well as some shot for research purposes were collected to provide additional information on formation, regression and persistence of various luteal structures. This sampling also was carried out in close cooperation with local wildlife boards and municipal authorities.

Age determination

The age of all females in this study was known either from marking or from sectioning decalcified roots of first incisors (Reimers and Nordby 1968). Each age class runs from 1 June year N to 31 May year N + 1, inclusive.

Fixation and staining

Ovaries were either frozen temporarily and later fixed in 10% formalin, or fixed in 10% formalin directly. A special sample of ovaries was fixed in Bouin's fluid.

Three staining procedures were applied, – Haemalun-Eosin, van Gieson, and Heidenhains iron-haematoxylin (Romeis 1949; Gabe 1976). The latter was used primarily in routine analysis, the others for comparative work and more detailed studies of structural components.

Sectioning of ovaries

For a preliminary, macroscopic examination, ovaries were sliced sagittally with a scalpel into approximately 2 mm sections. The sections were backlighted with optical fibre illumination and examined for histological structures through a dissection microscope (4–20 x). The sections were then alternately freeze-sectioned (15 micron) at approximately 100 micron intervals and sectioned after parafian embedding (5–10 micron) correspondingly.

The microscopic slides produced represented serial sections with combinations of different fixation procedures, sectioning techniques and staining, thus providing possibilities for a comparison of processing procedures.

Analysing procedure

Analysis of reproductive organs comprised the following steps:

1. External examination of size and appearance of uterus indicated parous or non-parous status of the hinds. A selection of trimmed uteri was weighed and described anatomically (Langvatn 1992).
2. Pregnancy or non-pregnancy was assessed from presence or absence of embryonic or fetal tissues in the open-slit uterus following the procedure described by Markgren (1969).
3. Ovaries cut free from the mesovarium at the hilum were weighed separately and examined for surface scars, indicating recent and previous ovulations. Extruding corpora lutea were also recorded.
4. Macroscopic examination of the gross sections of ovaries revealed luteal structures and pigmented scars. When possible, these structures were related to surface scars (Kelly and Challies 1978). The diameter of the largest Graafian follicles and luteal structures were measured in complete pairs of ovaries (Lea-

Tab. 1. Some characteristics of luteal structures and their regressing stages in ovaries sampled in September–November. See also text for description of the structures.

Structure	Diam. (mm)	Colour	Outline and shape	Ovulation rupture	Lutein cells	Blood vessels	Connective tissue	Hyaline material
PCL	4–10	Pale yellow, yellow-grey. Haemorrhagic exudate occur.	Regular. Body round or oblong. PCL < 6 days with central lumen and lutein tissue lining the follicular wall.	Obvious, but often healed. Recent ruptures sometimes surrounded by coagel-filled capillaries. Surface depression or ring-shaped extrusion.	Polygonal or oval graunulosa lutein cells transitional or developed (25–30 micron). Smaller theca lutein cells mostly in periphery and trabecular. Cell membranes diffuse, cytoplasma light.	Capillaries infiltrate luteal tissue. Larger vessels in radial trabeculae and the external thecal layer.	Sparce and inconspicuous except in external thecal layer.	Inconspicuous or absent.
CLS	2–8	Pale yellow-grey to light orange.	Often wrinkled, disintegrated. Body irregular and diffuse.	Always healed and non-haemorrhagic, no coagel. Usually obvious as a surface depression.	Irregular, necrotic lutein cells scattered and peripheral. Cytoplasma vacuolated and progressively granulated. Cell contours diffuse, increasing pigmentation. Intercellular space irregular.	Degenerative changes in vessels, pycnosis of endothelial cell nuclei. Capillaries mostly in periphery, otherwise as in PCL.	Trabecular strands and increasing amounts of less structured tissue.	Scattered, mostly in central areas.
CLV	11–16	Cream yellow, yellow-grey. No haemorrhagic exudate.	Like PCL, but body larger. No central lumen. Distinct outline.	Like CLS. May also protrude on the surface as a ring-shaped ridge.	Cells (granulosa origin) larger than in PCL, rounded or oblong with pointed ends (35 micron). Cytoplasma homogenous or finely granulated with large transparent nuclei. Cell membranes distinct, intercellular space negligible. Fewer theca lutein cells than in PCL.	Like PCL. Vascularization rich.	Like PCL	Like PCL
CLA	2–5	Like CLV	Regular when body dense, round or oblong. Outline often folded when	None	Lutein cells smaller (30 micron) than in CLV, otherwise similar.	Like PCL, perhaps fewer large vessels.	Sparce and inconspicuous.	Like PCL

			contains a central lumen.					
CLPC	5–10	Like PCL	Like PCL. Body may be flattened or distorted.	Like PCL. Appear more recent than in CLV of the same ovaries.	Like PCL or CLV	Like PCL or CLV	Like PCL	Like PCL
CR	2–4	Bright orange to reddish-brown.	Partly diffuse, body fairly regular and continuous, but may be flattened or distorted.	Always healed, often visible as a surface depression, or as a pigmented protrusion.	Decreasing in number with regression. Cells with lipid vacuoles, and fragmented. Cytoplasma increasingly granulated, nuclei pycnotic and opaque. Lutein cells pigmented. Pigment crystals scattered in connective tissue. See also CLS.	Capillaries degenerating, lumens collapsed and endothelial cell nuclei pycnotic. Larger vessels, mostly peripherally and between strands of connective tissue also degenerating.	Conspicuous, diffuse outer capsule and trabecular strands in early regression. Later completely enveloping vascular elements and degenerating lutein cells.	Scattered, mostly in central areas in early regression.
CA	<2	Pale greyish-white, sometimes brown.	Diffuse and difficult to define. Scar tissue discontinuous.	Always healed, inconspicuous, usually difficult to detect and associate to scar tissue.	Lutein cells (if any) are few and highly degenerated. Cell debris and pigment granula wedged between connective tissue cells.	Few capillaries and finer vascular elements, degenerated and scattered. Larger, thickwalled vessels in scattered clusters.	No connective tissues capsule. Stroma-like tissue diffusely marking the structure, identified from clusters of vascular elements and debris from degenerated lutein cells.	Sparce, usually absent.
CAA	<3	Like CR, but usually less brightly pigmented.	Like CR	None	Like CR, but less pigmented lutein cells.	Like CR, but fewer large vessels. Vascular elements mostly peripheral.	Like CR. Outer capsule inconspicuous and diffuse. Connective tissue perhaps more conspicuous in central areas.	Like CR

der-Williams and Rosser 1983). A simple drawing was made of position, size and appearance of ovarian structures in each section, thus building up a three-dimensional sketch of the organ.

5. As a last step, microscopic slides were examined to identify and describe luteal structures of different origin and regressing stages. Also, atretic follicles were recorded.

Each step in the analysis was performed blind, i.e. without reference to data on age, sampling date, lactational status etc., or knowledge of the outcome of preceding analysing steps for a particular animal. Only after the final examination of microscopic slides were data from different stages of the analysing procedure compared, and a final reproductive status recorded (conf. Morrison 1960; Leader-Williams and Rosser 1983).

Comparative processing and analysing procedures

To examine the success of different processing procedures in detecting and identifying ovarian structures, a sample of ovaries from 42 parous hinds with known reproductive histories was divided into two main groups. One group comprised pairs of ovaries preserved by freezing and/or subject to freeze-sectioning, and the second group without freeze-treatment at any stage during processing. Pairs of ovaries initially fixed in formalin were cut sagittally in approximately 2 mm blocks which alternately were freeze-sectioned and sectioned after embedding in parafin. Thus ovaries from the same individuals occur in both groups in treatment. Seven pairs of ovaries were frozen directly and treated with formalin only for hardening before sectioning.

Within the two groups each set of ovaries was examined both macroscopically and microscopically. From the background of reproductive events known in each hind a "reference" distribution of structures (PCL, CLS, CLV, CR, CA) was obtained (Tab. 6). Structures that could not be anticipated from the reproductive history and status of the individuals (CLA, CAA) were excluded from this comparison (Tab. 6).

Results and discussion

Morphometry and occurrence of ovarian structures

Mean size and range of ovarian structures on a yearly basis (Tab. 2) correspond fairly well to those given by other authors (see above). Minor deviations in maximum values for CLA, CR, and CAA are probably due to this study's larger sample size and access to ovaries from a period when these structures were at a maximum developmental stage (Morrison 1960). It should be emphasized that except for PCL, CLV, and CLA, the outline and size of other structures were often irregular and difficult to define, hence size figures are merely indicative, especially in the lower range of values.

Tab. 2. Mean diameter of ovarian structures as an average for all age classes sampled throughout the year.

Structure	Mean (mm)	± S.D.	Range (mm)	N
PCL	7.7	1.11	4 - 11	684
CLS	3.4	1.18	2 - 6	46
CLV	10.8	1.79	6 - 15	114
CLA	4.2	0.89	2 - 8	213
CR	2.5	0.62	1 - 9	1471
CA	1.1	0.34	1 - 2	856
CAA	1.7	0.56	1 - 4	235

Mean size of luteal structures varied with month of sampling (Tab. 3), but not with age of the hinds (one-way analysis of variance, Scheffe's procedure, all $p > 0.05$). Langvatn (in prep.) has shown that ovaries tend to increase in size as hinds grow older, up to approximately 12 years, and since large structures like PCL and CLV often constitute a large proportion of the ovary (Halazon and Buechner 1956; Markgren 1969), one would predict an association between the size of these types of corpora lutea and the weight of ovaries, as shown in reindeer (Leader-Williams and Rosser 1983) However, albeit a certain trend in that direction for CLV, covariance between these luteal structures and weight of ovaries was not significant (analysis of variance, regression approach model, PCL: $F_{1,477}0.438$, $p = 0.51$, cov. raw regr. coeff. = 0.04, CLV: $F_{1,96} = 2.519$, $p = 0.11$, cov.raw regr. coeff. = 0.39). Valentincic (1958) suggested that yearling hinds had smaller corpora lutea due to their small ovaries, otherwise he too found no relationship with age of the females,

contrary to what Markgren (1969) indicated for moose.

The mean diameter of luteal structures changed with date (Fig. 2). The PCL showed a rapid increase in mean diameter with time, and by November a significant proportion of the PCL's measured were equal in size to many CLV. This probably means that many PCL were virtually CLV, and that sampling took place near, but prior to the stage when an implant could be detected macroscopically. The interval from ovulation to full formation of PCL is normally 8–10 days in many bovids and cervids (McDonald 1975; Nalbandov 1976). In case of conception, it takes another 10–15 days before an implant is visible (Valentincic 1958; Markgren 1969; Nalbandov 1976).

In white-tailed deer, Trauger and Haugen (1965), Brokx (1972) and Harder and Moorhead (1980) have demonstrated a gradual increase in the corpus luteum from ovulation throughout pregnancy. Morrison (1960) reported growth of CLV in elk until the 70th day of gestation, whereafter there was considerable variation in diameter. In red deer, Valentincic (1958) suggested that CLV has its maximum size during the first month of pregnancy, and that there is a decrease in size during the succeding two months. However, in this study it is evident

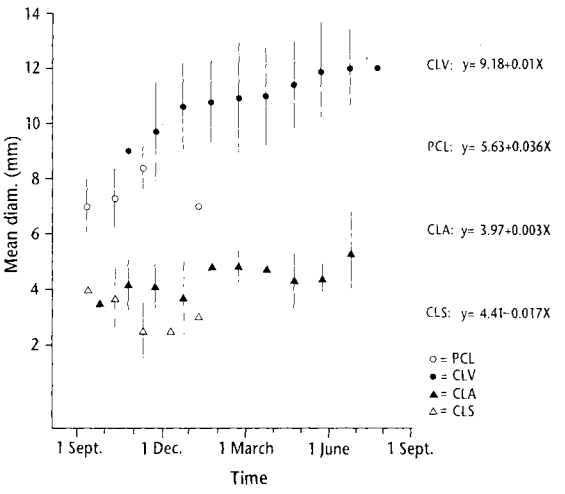

Fig. 2. Change in size of luteal structures with time. Plots are monthly means ± S. D.

that CLV increase in size beyond the first three months of gestation (Fig. 2). Furthermore, it is also likely that in the case of conception, the growth pattern of corpus luteum from ovulation to parturition is non-linear and asymptotic at around 12 mm mean diameter (see Morrison 1960, Fig. 1 p. 303; Markgren 1969; Kelly and Challies 1978).

The final size of the CLV is related to the diameter of the mature follicle from which it

Tab. 3. Relationships between size of luteal structures (mm) and month of sampling (analysis of variance).

	PCL			CLV			CLS			CLA		
	$\bar{x}$	±S.D.	N	$\bar{x}$	±S.D.	N	$\bar{x}$	±S.D.	N	$\bar{x}$	±S.D.	N
Sept.	7.0	0.95	33				4.00	1.41	2	3.5	0.71	2
Oct.	7.3	1.06	430	9.0	0.00	2	3.73	1.11	30	4.2	0.79	97
Nov.	8.4	0.82	220	9.7	1.76	27	2.50	0.97	10	4.1	0.87	77
Dec.				10.6	1.57	21	2.50	0.71	2	3.7	0.76	7
Jan.	7.0	—	1	10.8	1.54	11	3.00	1.41	2	4.8	1.26	4
Febr.				10.9	2.15	9				4.8	1.48	5
March				11.0	1.79	6				4.7	0.58	3
April				11.4	1.24	12				4.3	0.95	7
May				11.9	1.81	11				4.4	0.55	5
June				12.0	1.36	14				5.3	1.51	6
July				12.0	—	1						
Aug.												
F	66.467			3.401			2.955			2.111		
d.f.	3			9			4			9		
p	<0.001***			0.001***			0.031*			0.03*		
R^2	0.227			0.227			0.224			0.086		

arose (Harrison 1948), and CLV can attain about twofold the size of the follicle (Harrison and Weir 1977). Mean diameter of the largest follicle in Norwegian red deer reported by Langvatn (1992) was 5.2 mm. Compared to 12 mm as mean maximum diameter for CLV (Fig. 2), this supports the observations of Harrison and Weir (1977). From the measurements summarized in Figure 2 it is clear that size per se is not always a reliable criterion in distinguishing between PCL and CLV, but may be corroborative when the time of sampling is known.

In the event of a released ovum not being fertilized and thus implantation of a blastocyst in the uterus not occurring, the PCL developed after ovulation commences rapid degeneration from day 12–15 of the cycle (McDonald 1975; Nalbandov 1976; Harrison and Weir 1977), and by definition becomes a CLS. Only 46 CLS were identified, and the large majority occurred in October and November (Fig. 2). The mean size of CLS declined rapidly from late September/October to November, ($t=3.13$, d.f.$=38$, $p=0.03$). Four individuals sampled after the main breeding period, however, had larger CLS than expected from the general trend in autumn. Interestingly, two of the four hinds were first time breeders (1 and 2 years old). A parous 2 year old hind dead in January was in extremely bad condition (dressed weight 30.0 kg) and nonpregnant. Her ovaries contained both a CLS and a PCL. The other hind also was dead in January, was 15 years old, and had a fetus of only 16 g.

Accessory corpora lutea (CLA) were frequently found from the start of the breeding season and throughout the pregnancy period. The size of CLA increased with date (Fig. 2), although at a rather lower rate than CLV (Kelly and Challies 1978). Consistent with findings by Douglas (1966), CLA were always smaller than PCL and approximately half the size of CLV (Kelly and Challies 1978). CLA were distributed evenly between the ovaries, irrespective of the position of the CLV (Leader-Williams and Rosser 1983).

Halazon and Buechner (1956) described secondary corpora lutea originating from postconception ovulations (CLPC). In this study, when additional luteal structures were found in pairs of ovaries containing a CLV, and the structures were associated with ovulation scars, the additional structures were always regressing luteal bodies (CLS). In other words, CLPC as discribed by Halazon and Buechner (1956) were not encountered in the present study (see also Trauger and Haugen 1965).

After parturition the former CLV (by then a CR) showed rapid regression to a mean diameter of 2–3 mm by autumn (Fig 3). Variation in size was considerable and often difficult to measure, probably as a result of variation in follicular activity and tissue dynamics in the ovaries (Golley 1957; Morrison 1960), and differences in time of parturition. For example in 22 hind-calf pairs shot between 16 and 30 October there was a negative relationship between size of CR in the hind's ovaries and dressed weight of her

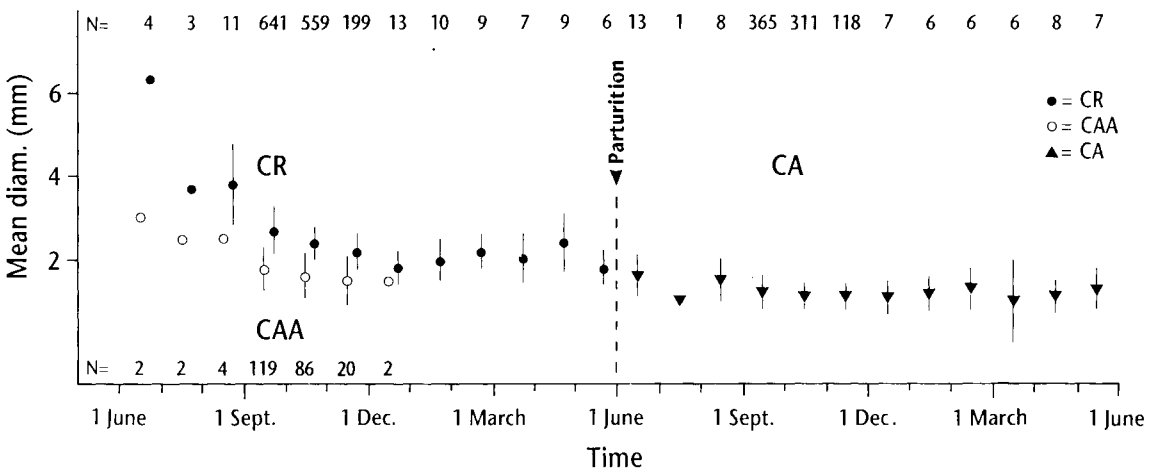

Fig. 3. Change in size of regressing luteal structures with time. Plots are monthly means ± S. D.

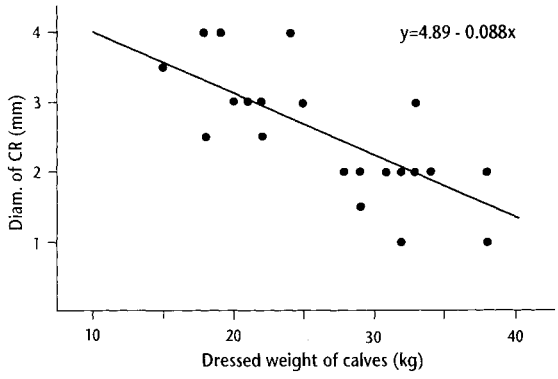

Fig. 4. Relationship between the diameter of CR in ovaries from lactating hinds and dressed weight of their calves.

calf ($R^2=0.48$, $F_{1,20}=18.116$, $p<0.001$, – Fig 4). Thus, if dressed weight in calves reflects variation in time of birth, large CR in autumn may be indicative of a late parturition and vice versa.

CA are smaller than CR (Fig. 3), but as mentioned it was often difficult to assign a defined outline and obtain realistic measurement of diameters (see also Leader-Williams and Rosser 1983). However, size and differences in histological appearance provide a basis for distinguishing between CA and CR, especially in early autumn. As shown below, CA may persist for years (Markgren 1969), but it is unrealistic to age different generations of CA (Morrison 1960; Mansell 1971) on the basis of either size or appearance. The regression of CR and the succeeding CA with time appears curvilinear, with lower asymptote for mean diameters of CA being < 1 mm.

Histologically, the CAA (corpus albicans derived from corpus luteum accessorium, – CLA) resembles a CR. When both structures were present in a pair of ovaries, the smaller was defined as the CAA (Fig. 3). CAA decreased in size from June ($F_{1,230}=37.08$, $p<0.001$, $R^2=0.139$, $y=2.77-0.008x$), and by September it overlapped in size with both CR and CA. Size alone was not always sufficient to distinguish between CA and CAA, but histological differences were generally clear.

Variation in size between types of different ovarian structures did not show any significant relationships. Contrary to findings by Kelly and Challies (1978), there was no difference in mean size of CLV, whether it occurred alone or together with one or more CLA in the same pair of ovaries ($t=-0.34$, d.f.$=112$, $p=0.73$).

Primary corpora lutea (PCL) occurred from late September to the end of November, indicating a rather long period of ovulatory activity among the hinds. Of hinds two years and older culled in September only 4.9% had a PCL, compared to 67.1% and 87.7% in October and November respectively. As mentioned above, some PCL in November were probably functional CLV, albeit an implant could not yet be detected. Size and appearance of uteri (Langvatn in prep.) supports this presumption. Hence in November, the actual proportion of PCL was somewhat lower and CLV correspondingly higher than showed in Figure 5. Considering the time required from ovulation until an implant can be detected macroscopically, it appears that late October and early November is the time when most hinds conceive, as substantiated by the increasing occurrence of CLV in November and December. Yearling hinds tend to start ovulation somewhat later than older hinds (see Mitchell *et al.* 1981).

Conception rates in Norwegian red deer hinds 2 years and older, are high (93–100%, – Fig. 5), compared to other European populations (Valentincic 1960; de Crombrugghe 1964; Mitchell 1973; Mitchell *et al.* 1981; Albon *et al.* 1983). Of a total of 10 non-pregnant hinds (2 years and older) that died between January and May, five were injured (shot at during the preceding hunting season). This may explain their barren status.

Judging from the number of CLS identified, few hinds seem to have recurrent estrous cycles

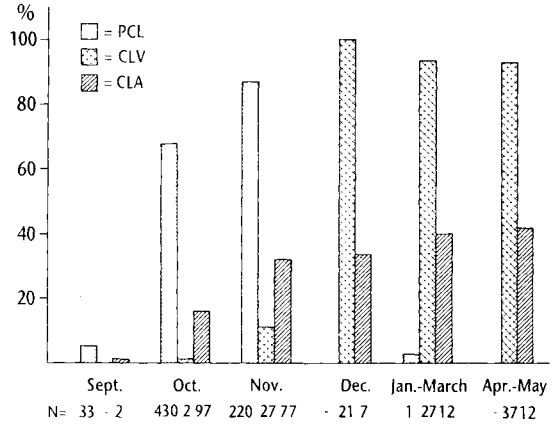

Fig. 5. Percentage of ovaries (pairs) from hinds ≥ 2 years, containing PCL, CLV and CLA.

before they conceive. This contrasts with black-tailed deer (Thomas and Cowan 1975) and white-tailed deer (Harder and Moorhead 1980), where most females conceive in their second cycle. In this study only 4.7% of all individuals examined in different months (range 0.3–5.8%) had a CLS, and there was no particular trend in this proportion with time.

Accessory corpora lutea (CLA) were found on average in 20.2% of pairs of ovaries examined. Similar figures reported by Valentincic (1958), Douglas (1966), Guinness *et al.* (1971) and Kelly and Challies (1978) were 33%, 37%, 52% and 16.3–58.9% respectively. Kelly and Challies (1978) showed an increase in occurrence of CLA from onset of the rut to later stages of pregnancy. That was also the case in this study (0.4–42%, Fig. 5). A log-linear model ($y = a + b$ (lnx) of the monthly proportions explained 98% of the variance, $F_{1,5} = 176{,}684$, $p < 0.001$. The rate of development of CLA is greatest in early pregnancy, but quickly asymptotes (Fig. 5), suggesting that the structures probably remain until parturition (Halazon and Buechner 1956; Kelly and Challies 1978).

In *Perissodactyla* accessory corpora lutea become frequent about 40 days after ovulation (Harrison 1948). For elk Halazon and Buechner (1956) report CLA to develop at the time the embryo becomes macroscopic. Incidence and distribution of CLA in the present study seem to substantiate this opinion. Also Kelly and Challies (1978) suggest that CLA is formed within two months of the breeding season. Possibly with one exeption, CLA were always found together with a PCL or CLV, and not with a single CLS. Hence, if CLA is likely to develop primarily in hinds that have conceived, and at the time of implantation, this also supports the suggestion that some PCL acually were functional corpora lutea of pregnancy. Further, since much of the sampling in this study took place early in the breeding season, this may in part explain the low average percentage of hinds with a CLA compared with other investigations (see above).

The actual role of CLA in red deer is obscure since pregnancy is adequately maintained in hinds without a CLA in the ovaries (Kelly and Challies 1978). Harder and Moorhead (1980) showed a significant correlation between total volume of luteal tissue and levels of circulating progesterone in white-tailed deer. Thus one possible prediction is that the CLA occurs as a "supporting" structure to enhance the progestogenic activity, and that a CLA is more likely to occur when the CLV is small. However, such a relationship could not be demonstrated in this study (see above). Furthermore Kelly and Challies (1978) found the predicted trend reversed.

Contrary to Morrison (1960) the occurrence of a CR in this study proved to be a reliable criterion for parturition during the preceding summer. This was verified by the lactation status of the hind. In all lactating hinds a CR could be identified up to the end of November. Also in hinds (2 year old) that had given birth (judged from the size of uterus, – Langvatn in prep.), but lost the calf neonatally and ceased lactation, a CR could easily be identified at least throughout October (N=4). By November the CR had degenerated both in size and appearance to a degree that required more attention to distinguish it from «large and conspicuous» CA. Distinguishing CR from CAA was never a problem, since by definition CR was always the larger when the two histologically similar structures occurred together.

As a consequence of the definitions used, CAA should occur in the same proportion to CR as CLA to CLV, provided that CR and CAA have the same probability of being discovered and identified at a given time post partum. However, that was not the case, the ratios being 0.16 and 0.42 respectively (deviation from the expected frequency: $X^2 = 220.0$, d.f. = 1, $p < 0.001$). One possible explanation for this could be that since CLA are approximately half the size of CLV, and assuming comparable rates of degeneration of CAA and CR, the post partum regression may have reached the size and appearance of CAA by late autumn that then resulted in confusion with CA. It could also be that CAA, being generally small, degenerated to an extent that significantly reduced the possibly of detection alltogether.

Histologically, PCL, CLV and CLA show many similarities at the cellular level, and they may be difficult to distinguish between unless additional criteria are considered (see description above). This includes association with an ovulation rupture (PCL, CLS, and CLV, – Fig. 6), presence of a conceptus (CLV and CLS) and a central, non-luteinized lumen (only in young PCL and occasionally CLA). Since the developing PCL is fully luteinized after approximately

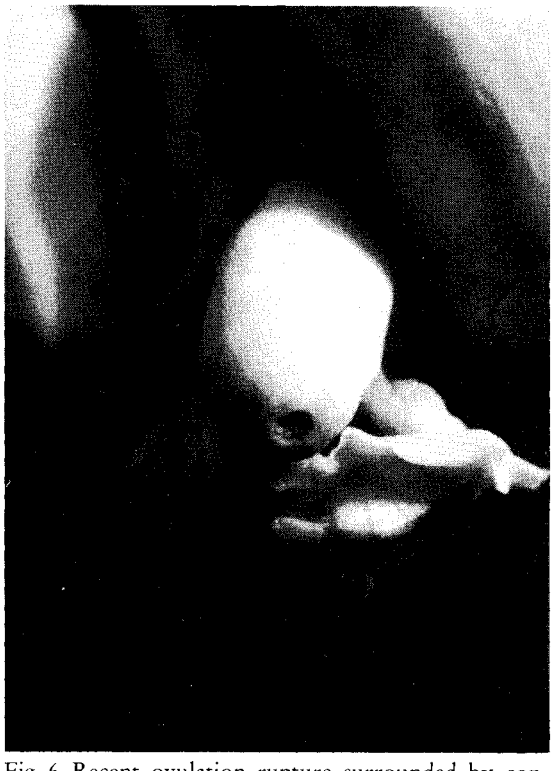

Fig. 6. Recent ovulation rupture surrounded by conspicuous blood vessels.

one week (Harrison 1948; McDonald 1975; Harrison and Weir 1977), with no central acavity left, this provides additional, useful information on the time of ovulation (Haagenrud and Markgren 1974). Size per se is not a reliable criterion, but all non-regressing luteal structures <4 mm were CLA, and those >9 mm were all CLV.

CLS were usually easy to identify histologically from signs of degeneration. At more advanced stages of regression, light yellow-orange pigmentation was noticeable on most occasions. Pigmented CLS were observed for example in two recycling yearling hinds, thus excluding any possibility of confusion with CR or CAA, and supporting the suggestion that all luteinized structures probably show pigmentation upon regression (Golley 1957; Morrison 1960; Markgren 1969; see also Cheatum 1949). However, the results of this study indicated that CLS probably do not attain the same degree of pigmentation as regressing CLV and CLA (Trauger and Haugen 1965). Also, CLS probably regress faster and become less conspicuous than other luteal structures (Brokx 1972). Halazon and Buechner (1956) reported similar observations, while Morrison (1960) claimed that CLS were more intensively pigmented than degenerating CLV. However, also Morrison (op.cit.) indicated a fast regression rate for CLS. There is no information in the literature indicating that CLS can be traced for more than approximately 2 months after its formation. Consequently CLS should not be confused with CA in samples from winter and spring. However, in autumn (October and November), CLS may be mistaken for either a CR or CAA (Leader-Williams and Rosser 1983), even though a recent CLS (<20 days post ovulation) is likely to be larger in size and show more signs of early degeneration. Also, in practice the occurrence of a CLS is so infrequent that it does not represent a quantitative problem of any significance in reproduction studies of red deer. Histological appearance of various luteal structures and their regressing stages is illustrated in Figures 7–13.

Retrospective studies of cervid reproduction and fecundity rely on identification of regressed luteal structures (Morrison 1960). Consequently, ageing characteristics, longevity and positive differentiation of the structures as to origin are a crucial question in the analytical process (Golley 1957; Mansell 1971). Definitions and characteristics of ovarian structures used in this study are appropriate for the purpose of indicating recent reproductive events. Depending on the questions asked, the usefulness of the approach can be further enhanced by careful selection of sampling periods. For example, retrospective assessment of natality rates should preferably be made on ovaries sampled in early autumn (September), prior to the main period of ovulations. That will reduce the possibility of confusion between CR (CAA) and CLS. Also, in September CR will have retained more of the characteristics distinguishing it from CA (Thomas 1983), compared to late autumn. High follicular activity and development of luteal structures increases the risk of distortion of CR, CAA and CA, and probably reduces the possibility to detect and identify these structures (Markgren 1969). Although not a serious problem in this study, more effort and accuracy seems required to detect and identify CR, CAA and CA in ovaries containing large luteal bodies which sometimes more or less "enclose" the pigmented scars.

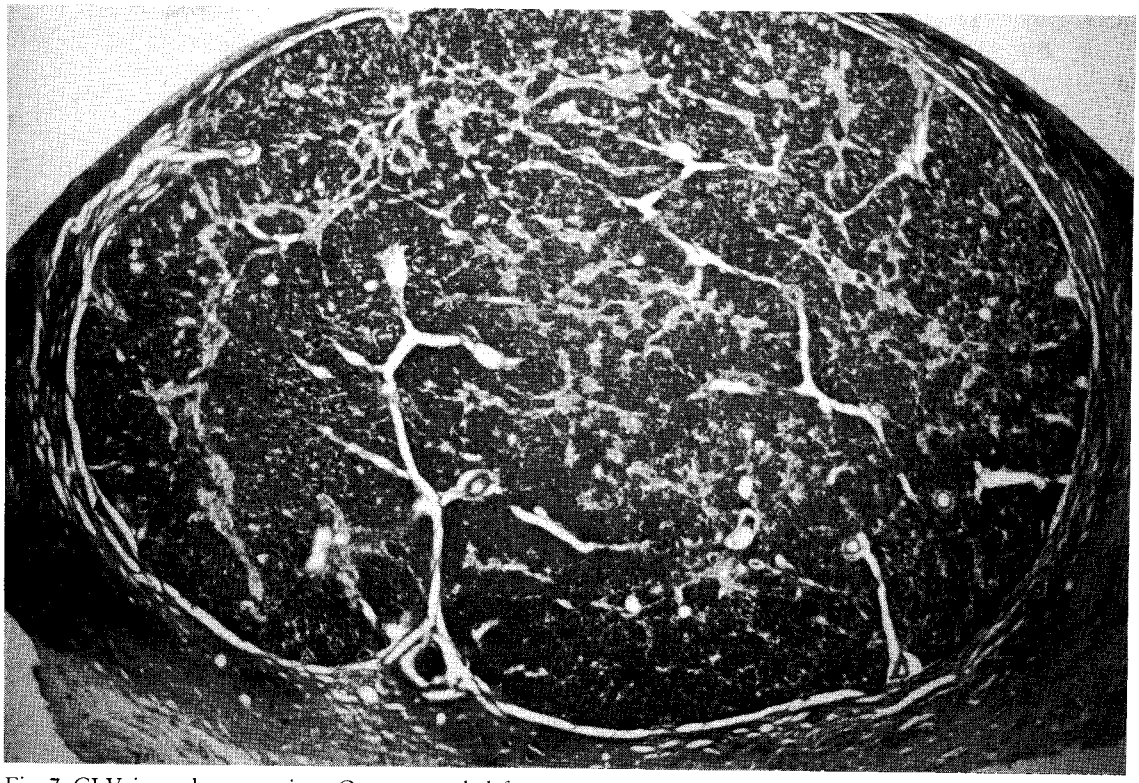

Fig. 7. CLV in early regression. Ovary sampled from a pregnant hind June 5, immediately before term. The hind had a developed udder and a fetus weighing 7,9 kg.

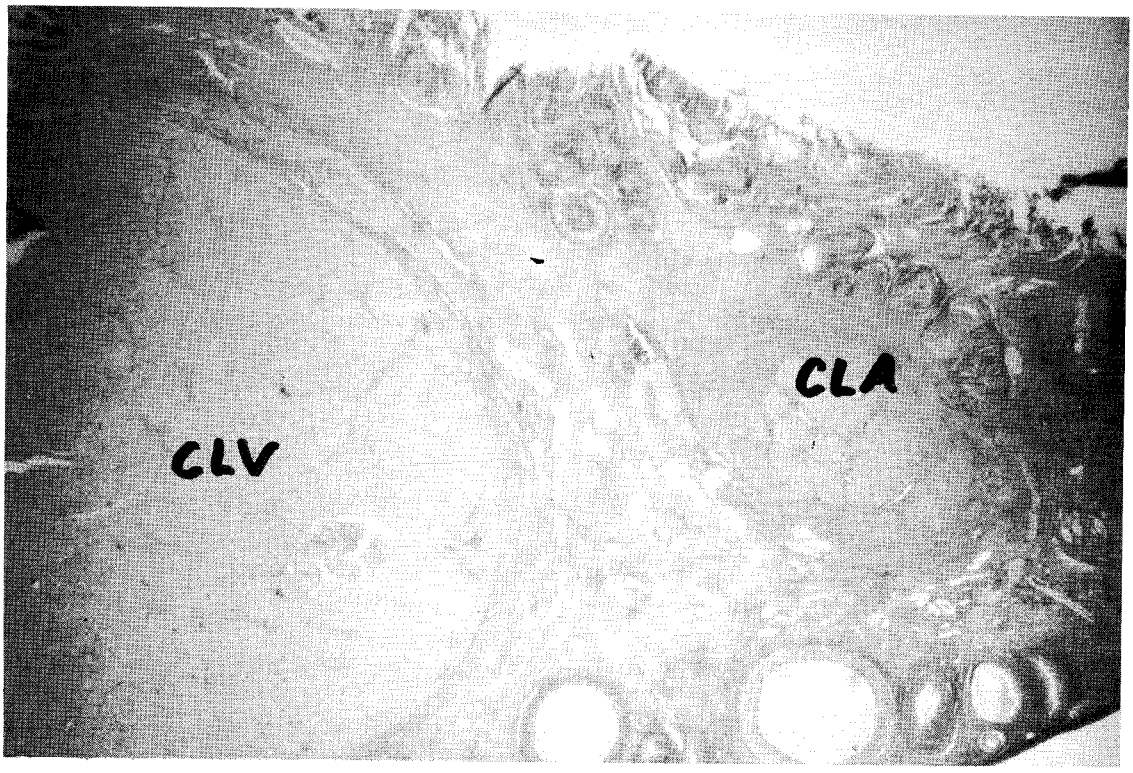

Fig. 8. CLV and CLA in ovary from a 2 year old hind sampled December 20.

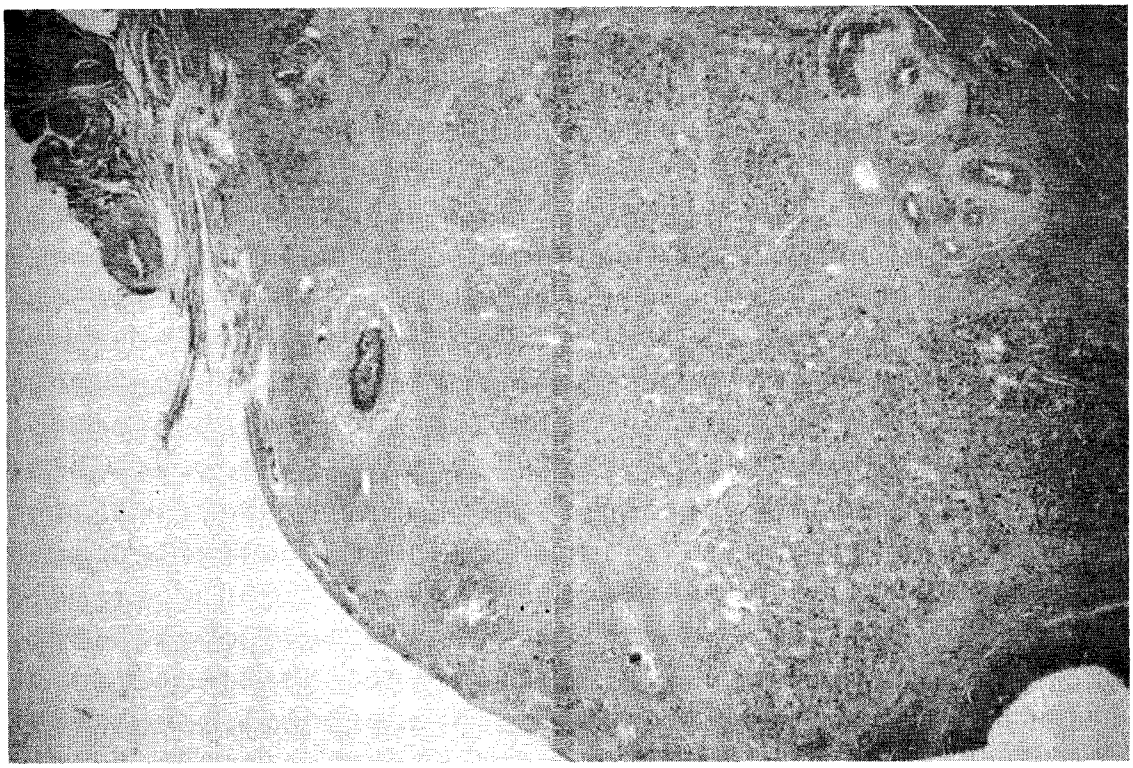

Fig. 9. CLS in early regression. The hind had ovulated 13–14 days prior to sampling.

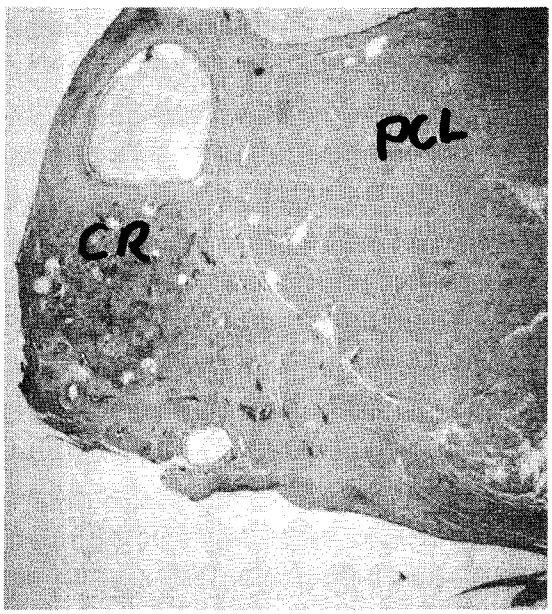

Fig. 10. PCL and CR in ovary from an adult hind sampled October 22.

Investigation of conception rates can be carried out in late autumn (from November), using the occurrence of luteal structures (CLV). This should be more reliable than analysis based on CR alone, especially in multiparous individuals. However, the two approaches are best used in combination, to extend the total information gained from each individual. Ovulation rates and timing of estrus can be assessed adequately from PCL, while occurrence of CLA may provide supporting indication on conception periods assessed from the ratios of PCL and CLV.

In theory, surface ovulation scars may be of help in estimating the number of ovulations, but there is a problem with the persistence and detection of these scars as the season progresses. A maximum of 11 ovulation scars was found in pairs of ovaries in this material. Detection of ovulation scars with time appears even more difficult than for CA, and these scars probably serve their best function as supplementary characteristics to the set of structures defined above.

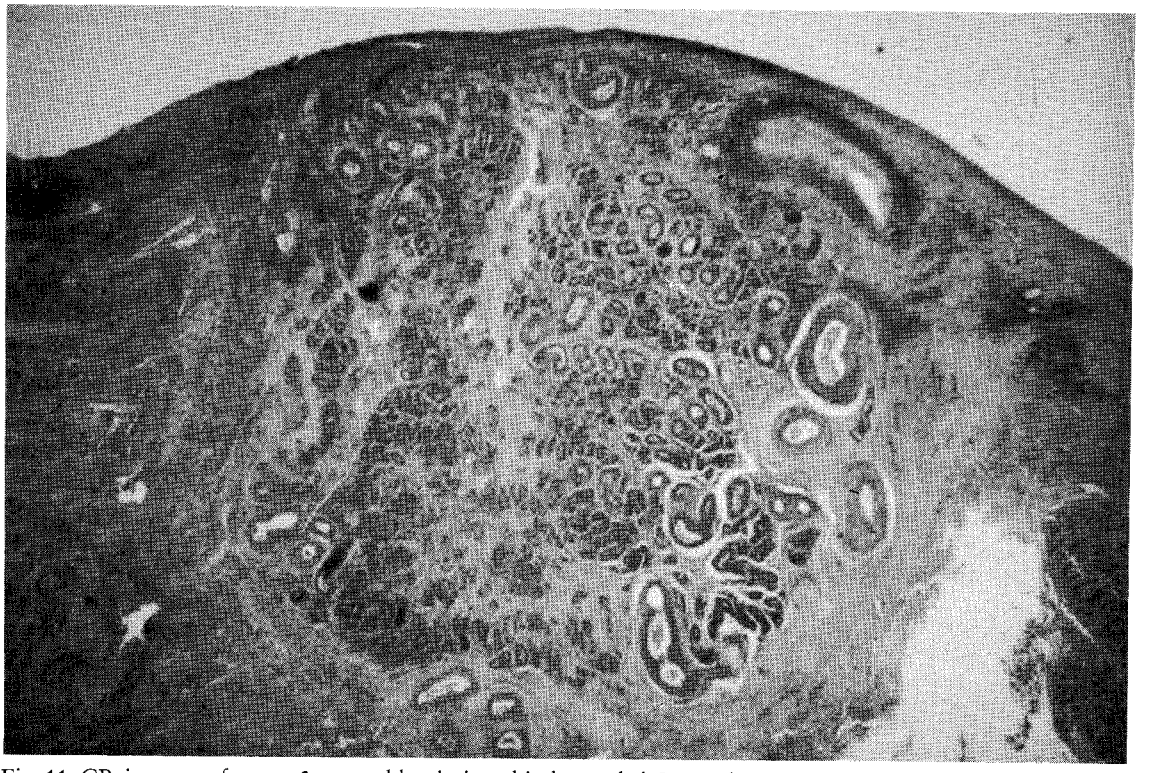

Fig. 11. CR in ovary from a 3 year old primipar hind sampled September 16.

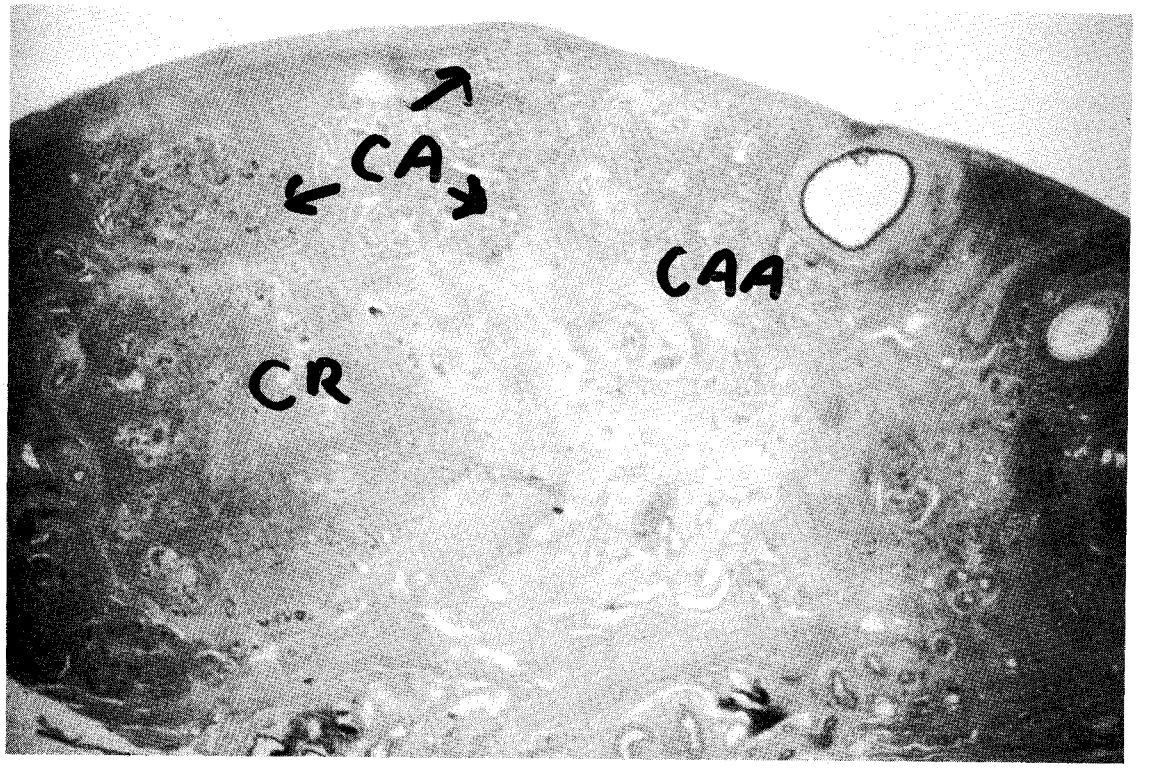

Fig. 12. CR, CAA and CA in ovary from a 13 year old lactating hind sampled July 23. Mean diameter of CR and CAA was 5.2 and 2.4 mm respectively.

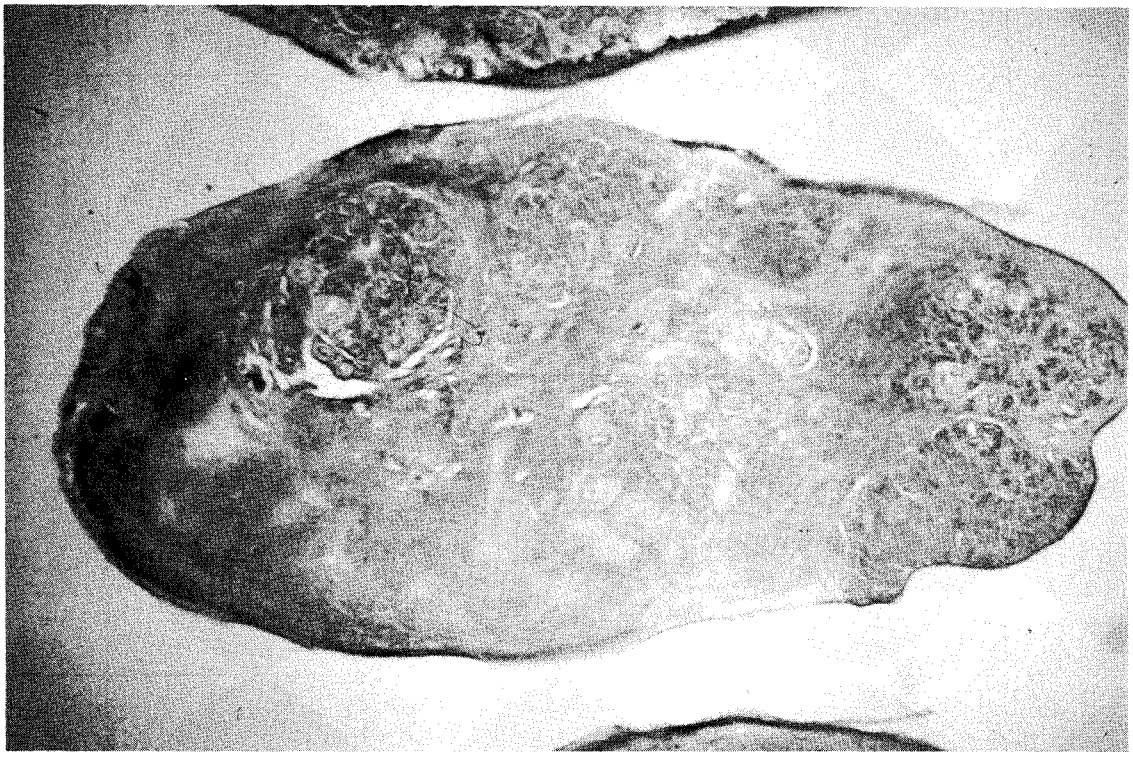

Fig. 13. Five CA identified in the left ovary of a 22 year old hind sampled October 10. The right ovary contained four CA but no CR and the hind was not lactating. No antral follicles were encountered in any ovary, probably indicating that the hind was in menopause.

Structural content in ovaries from hinds subject to controlled reproduction

Some background information on the hinds whose reproduction was closely monitored is summarized below (Tab. 4). Structures identified in the ovaries from these hinds are shown in Table 5.

Description of case-studies

Hind A: Age: 6 years. Date of death: 18 December 1975. Two CLS were found, one in each ovary. The largest, presumably 13 days old (post ovulation) had a mean diameter of 5 mm, and was greyish-white in colour without any trace of pigmentation. Degenerative characteristics were few at this early stage of regression, but the outline was rather irregular and in places diffuse. Strands of connective tissue trabeculae penetrated the structure together with a few blood vessels (Golley 1957; Nalbandov 1975). The other CLS (approximately 31 days) was 2.3 mm in diameter and had a pale yellow-orange pigmentation, difficult to detect macroscopically. Its appearance at the cellular level corresponded well to descriptions given by Valentincic (1958), Morrison (1960), Harrison and Weir (1977) for advanced regression stages of this type of structure. In addition to the two CLS, a condensed, irregular area of connective tissue and hyaline material, with capillaries interspersed, was found. This was perhaps a third CLS. However, no trace of necrotic luteal cells or other characteristics could verify that. Also, no remnants of a CLS produced in the hind's first estrus that season were detected.

Hind A showed estrous behaviour each rut and presumably ovulated several times between the ages of 2 and 5 years. However, she was never mated, and no trace of these ovulations was found, perhaps indicating that persistence of regressed luteal structures in some way may be related to pregnancy.

Hind B: Age: 8 years. Date of death: 21 January 1981. One CLV was found in the right ovary, verifying pregnancy, but no CLS relating to the

Tab. 4. Background data on hinds subject to controlled reproduction.

Hind	Year of birth	Date dead	Age at first conception	Total number of offspring	Years barren after first gestation	Comments
A	1969	18 Dec. 1975	—	0	—	Never mated. Four estrous cycles recorded in 1975 (10 Oct, 29 Oct, 17 Nov, 5 Dec).
B	1973	21. Jan. 1981	3	4	—	Two estrous cycles in 1980 (2 Oct, 19 Oct). Mated and conceived at last estrus.
C	1977	18 Nov. 1980	3	0	—	Nulliparous, conceived at first estrus in 1980 (25 Oct).
D	1970	1 Dec. 1977	5	1	1977	Three estrous cycles in 1977 (14 Sept, 4 Oct, 23 Oct). Mated and conceived at last estrus.
E	1978	26 Sept. 1984	2	4	—	Lactating. Four concecutive parturitions.
F	1980	23 Nov. 1981	—	0	—	Immature, no estrous cycles.
G	1979	8 Dec. 1988	2	7	—	Two estrous cycles in 1988 (10 oct, 28 Oct). Mated and conceived at last estrus.
H	1976	12 Dec. 1988	4	6	1987, 1988	Two estrous cycles in 1988 (1 oct, 19 Oct). Mated and conceived at last estrus.

first ovulation 111 days earlier. In addition, a CLA was present in the left ovary. One CR and a CAA confirmed parturition and gestation the preceding year, while two CA revealed two out of the three parturitions prior to 1980.

Hind C: Age: 3 years. Date of death: 18 November 1980. One CLV relating to a blastocyst was the only structure found. There was no indication of any ovulation in 1979.

Hind D: Age: 7 years. Date of death: 1 December 1977. The hind was pregnant with a small embryo, relating to a large CLV (11 mm diam.). One CLS was very irregular and diffuse in contour and approximately 1.7 mm in mean diameter. It was slightly pigmented (yellow-orange), but clearly less conspicuous than most CR of similar size. The pigmented areas with highly necrotic luteal cells were emeshed in connective tissue. Small capillaries occurred mostly in the perephery of the structure. What was presumed to be a second CLS consisted of connective tissue and a few capillaries indicating the periphery of a colourless structure (diam. 1 mm), similar to the possible third CLS described for Hind A. This structure could only be detected after detailed microscopic examination, and from the knowledge that the hind had recycled twice. A well defined CA (2.2 mm

Tab. 5. Structures in the ovaries from 8 hinds subject to controlled reproduction.

Hind	PCL	CLS	CLV	CLA	CR	CA	CAA
A	0	2(3)	0	0	0	0	0
B	0	0	1	1	1	2	1
C	0	0	1	0	0	0	0
D	0	2	1	0	0	1	0
E	0	0	0	0	1	3	1
F	0	0	0	0	0	0	0
G	0	1	1	0	1	6	1
H	0	1	1	1	0	2	0

diam.) corresponded to this hind's only parturition in 1976. This CA appeared surprisingly large and conspicuous considering it was 21 months old.

Hind E: Age: 6 years. Date of death: 26 September 1984. One CR and three CA were found, reflecting all four concecutive parturitions (1981–1984). In addition one CAA occurred. Since this hind was sampled prior to the breeding season, there were no signs of ovulatory activity in ovaries.

Hind F: Age: 1 year. Date of death: 23. November 1981. Immature yearling hind. Only follicles less than 3 mm in diameter were found in the ovaries of this hind.

Hind G: Age: 9 years. Date of death: 8 December 1988. Pregnant with a small fetus associated with one CLV (10 mm diam.). There were no convincing signs of the first estrus 58 days earlier. One CR and three CA in each ovary reflected all seven parturitions.

Hind H: Age: 12 years. Date of death: 12 December 1988. One large CLV (13 mm diam.) relating to a conceptus was identified. In addition, one CLA occurred in the same ovary. A small, diffuse and inconspicuous structure, possibly a CLS from the first estrus 62 days earlier was discovered (see hind A and D above). Of the six calves born to this hind, only two parturitions were reflected by the number of CA. The analysis missed four other CA, and therefore it was not possible to know which of the six parturitions were related to the CA positively identified. This hind had been kept barren for the two years preceding the last conception, but it is not known if this had any influence on the difficulties in detecting all the earlier pregnancies.

The attempt to relate ovarian structures to reproductive events in the eight hinds with known life histories indicates some of the limitations of the techniques applied. As expected, a CLV was found in all pregnant hinds carrying a visible conceptus. Also, a CR was identified in all hinds that gave birth during the immediate preceding summer. The number of CR and CA indicated the correct number of calves born to six out of eight hinds (nulliparous yearling included). However, for the other two hinds (B and H) the number of CR + CA counted were less than number of parturitions (1 and 4 respectively).

Observations on CLS support reports by Harrison (1948); Morrison (1960); McDonald (1975); and Nalbandov (1976) that CLS regress rapidly and are difficult to identify after 2–3 succeeding cycles. Some confusion between CLS, CAA, and perhaps also CA may occur in samples obtained in late October–November. The impression is however, that in CLS and CAA fewer and smaller blood vessels intersperse the structures, and they occur especially in the periphery. In CA, blood vessels and connective tissue are generally more conspicuous than in CLS and CAA, and CA have few, if any, remnants of necrotic luteal cells. A CLS is difficult to identify macroscopically, and smaller structures can only be distinguished in proper histological sections. Development of better criteria and procedures for retrospective analysis of red deer reproduction would undoubtedly be of great value both in ecological research and management.

Appraisal of processing and analysing procedures

The fixatives used in this study satisfactorily penetrated and fixed the different types of cell material. Ethanol (70%, – tried initially) seemed less suitable because it tended to bleach pigmented scars and hardened the tissue. The staining procedures applied discriminated well between structural components in the ovaries, and showed a good affinity for a wide spectrum of tissue material of which hyaline and connective tissue is particulary important for accurate identification.

The success in accurate identification of ovarian structures varied with processing and analysing procedure (Tab. 6), and some general experience from the comparison can be summarized as follows:

1. Freeze treatment of ovaries tended to disrupt cells and tissues, thus reducing the detection rate and correct identification of ovarian structures (see Schwartz and Diller 1980, – Fig. 14).
2. At the macroscopic level freeze treatment caused negligible difference to the efficiency and accuracy of the analysis.

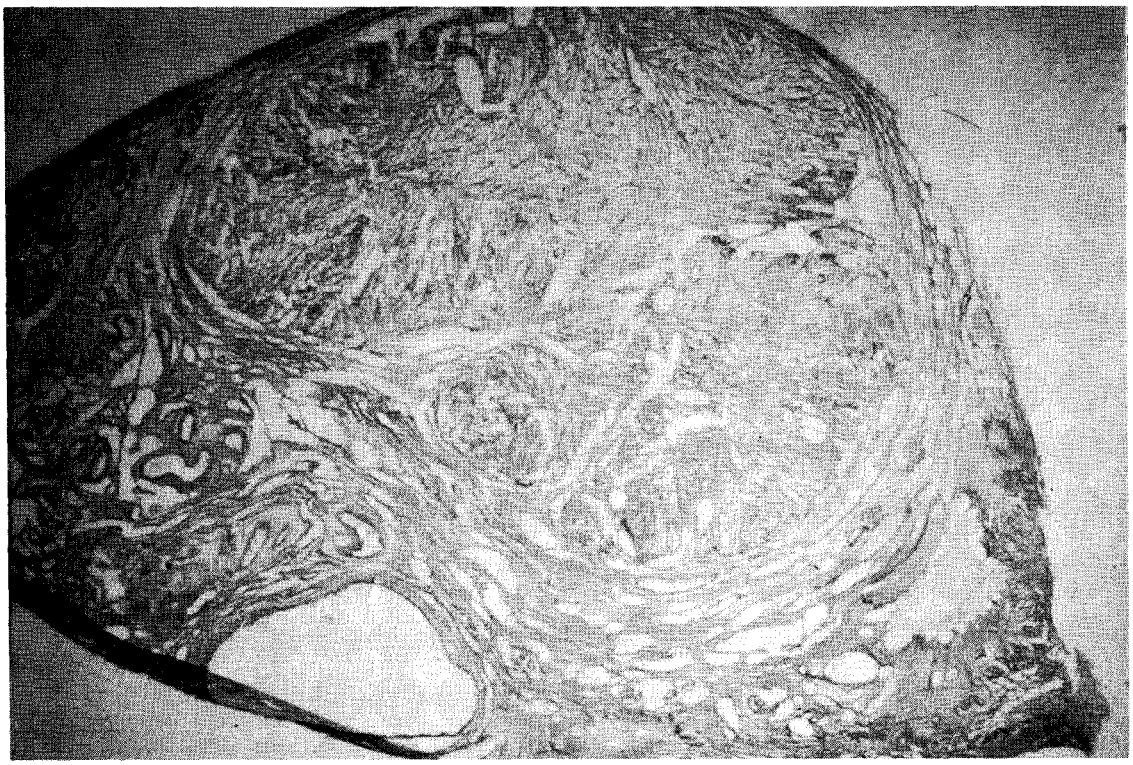

Fig. 14. Ovarian tissue disrupted by freeze-preservation.

3. Macroscopic examination of ovaries seemed adequate for the purpose of assessing ovulation (PCL) and/or conception (CLV), but may fail to distinguish PCL from early regression stages of CLS.
4. Macroscopic examination seemed adequate in detecting structures associated with parturition during the preceeding 5–6 months (CR).
5. Macroscopic examination of ovaries failed to detect and identify a significant proportion of the CA present, and also tended to overlook or incorrectly identify CR relating to parturitions more than 5–6 months earlier.
6. Microscopic slides based on material subject to freeze-treatment do not seem to improve resolution and accuracy in the analysis compared with macroscopic examination.
7. The advantage of proper histological procedures is primarily that more and older CA can be identified. In addition, the regressing stages of CLS can be distinguished from PCL, CR, CAA and CA.

Morrison (1960) claimed that to be practically acceptable, analysis of ovaries should be based on criteria that worked on a macroscopic level. This study concurs with this requirement, provided the analysis is restricted to PCL, CLV and CR in the samples. However, the gross procedure seems practical and reliable only when applied with full knowledge of its limitations (Cheatum 1949). Unfortunately, it is not always clear that this is appreciated. Furthermore, there appears to be significant interspecific variations (see e.g. Harrison 1948; Halazon and Buechner 1956; Buss and Smith 1966; Wegge 1975; Lockyer 1987) that effect the reliability of different procedures.

For most management purposes there appears to be little gained by carrying out time-consuming and expensive histological procedures. On the other hand, proper histological procedures are necessary for detailed studies of structures, and it provides some potential for retrospectively unraveling an individual's reproductive history. Although identification of CA in microscopic slides of non-frozen ovaries may appear convincing in Table 6, the figures do not necessarily reflect the real situation. It is not known to what extent old CA remained undetected,

Tab. 6. The effect of freeze treatment of ovarian tissue with regard to analysis of structural content.

	Assumed structural content (see text)	Level of analysis			
		Macroscopic		Microscopic	
		Identified	Misinterpreted and/or missed out	Identified	Misinterpreted and/or missed out
Freeze-treatment (N=42)	PCL=16	13	3	14	2
	CLS= 4	1	3	2	2
	CLV= 7	7	—	7	—
	CR =42	42	—	40	2
	CA =98	52	46	55	43
No freeze treatment (N=35)	PCL=12	10	2	12	—
	CLS= 3	1	2	3	—
	CLV= 7	7	—	7	—
	CR =35	34	1	35	—
	CA =85	47	38	76	9

but were "compensated for" by incorrect inclusion of CAA, especially in their later regression stages. Also, it could be a potential problem that CA are disrupted by tissue dynamics in the ovaries, and that groups of characteristic tissue from the same structure are interpreted as separate CA.

Concluding remarks

In this paper an attempt has been made to define a set of important ovarian structures used in analysis of red deer reproduction. These structures are defined and described in an effort to clarify the varied and often inconsistent terminology encountered in literature in this field. The tabulation and detailed description of appearance, morphometry and histological characteristics for each defined structure will hopefully facilitate the understanding and application of this approach.

The analytical approaches described may elucidate many of the most essential reproduction parameters, provided access to material from the autumn breeding season. However, problems with accurate identification of structures influence both qualitative and quantitative assessments of reproductive events. The magnitude of this problem is clearly associated with the processing and analysing procedures applied, and it is important to restrict observations to what is realistic and reliable in terms of methodological approach.

In spite of the limitation indicated above, analysis of ovaries can be a valuable method in studies of cervid reproduction. However, more systematic work on both qualitative and quantitative aspects of ovarian structures could further enhance its signiticance in biological research.

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