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Aetiology and pathogenesis of the development of corpus luteum, corpus luteum with cavity, cystic corpus luteum and lutein cyst and their effect on the fertility of dairy cows

Theses of PhD dissertation

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Introduction

As a result of the intensive but unilateral selection of dairy cows in the last decades, in our day dairy farms with a milk production exceeding 10,000 kg/cow/year are no longer a rarity. Parallel to that, fertility problems have become common. Milk production can be economical only if fertility is sufficiently high (the economic loss due to reproductive problems is estimated at 160–320 EUR/cow/year). Low pregnancy rate can usually be attributed to inactive ovaries or ovarian cysts.

Aims of the study

The aims of my study were to examine the incidence, formation, pathogenesis and aetiology of fluid-filled ovarian phenomena (except follicles), and to use these data to clarify the relevant nomenclature.

Studies

Materials and Methods

Incidence of fluid-filled ovarian phenomena in a dairy farm with a milk production higher than the Hungarian average in 2008–2011

Between 1 January 2008 and 31 December 2012, I performed rectal ultrasound examinations on a dairy farm (milk production approx. 10,000 kg/cow/year) every other week with a Tringa linear

ultrasound machine (linear probe, 6–8 MHz). I examined the ovaries and uterus of postpartum cows (30–60 days after calving) and inseminated (28-60 Days) cows and heifers (28–60 days after insemination), and recorded the data of these examinations, together with other data, in the CATUS Database. Statistical analyses were carried out with Fisher's exact test and logistic regression.

Seasonal fertility differences in synchronised dairy cows: ultrasonic, metabolic and endocrine measures

In each examination period (summer: from June to August 2008; winter: from November 2008 to January 2009), 10 randomly selected Holstein-Friesian cows were examined on a dairy farm with high milk production (approximately 12,500 kg/cow/year). In both examination periods, cows were put on the Provsynch regimen 30–36 days after calving. Blood samples were taken once a week during the examination period (from 30–36 days post partum up to 30–35 days post insemination). From the 1st day post insemination (PI), rectal ultrasonography was carried out once a week to detect phenomena on the ovaries (Tringa linear), and the data were recorded in the CATUS Database. The following metabolic parameters were measured: plasma beta-hydroxybutyrate (BHB) and non-esterified fatty acid (NEFA), serum beta-carotene and ferric reducing ability of plasma (FRAP). Pregnancy diagnosis was carried out 30 days post insemination using a Pregnancy-Specific Protein B

(PSPB) test (BioPRYN® ELISA). ANOVA, linear regression and Wilcoxon rank-sum test were used for statistical analyses.

Endocrine and morphologic characteristics of fluid-filled ovarian phenomena

Biopsies and aspirations

Preliminary studies of samples taken by biopsy from ovaries collected at a slaughterhouse did not give sufficient information about the exact histological morphology of the structures; therefore, biopsy sampling was not used *in vivo*. After culling, fluid from fluid-filled ovarian phenomena (FFOP) was aspirated with a 18G needle and a 10 ml syringe (n=15). *In vivo* aspirations were carried out from 11 Holstein-Friesian cows with high milk production after local anaesthesia, with a special sampling system, an ultrasound machine (5–7 MHz mechanical sector probe) and a 65 cm long, 16G needle. Parallel to the aspirations, blood sampling was also done.

The concentrations of NEFA, progesterone (P4) and 17-beta-oestradiol (E2) were measured from aspirated and blood samples. Validation of P4 and E2 measurements in aspirated samples was based on different-sized follicles. ANOVA was used for statistical analysis.

Morphological (ultrasonic, macro- and microscopic examinations)

Ovaries were collected from 90 nonpregnant dairy cows at an abattoir within 4 h after death between July 2008 and December 2011. No ante mortem data regarding reproductive history were

available. All luteal structures with a visible ovulation papilla were designated as being ovulatory (Groups A and B), whereas those without an ovulation papilla were designated as anovulatory (Groups C, D and E). Ovaries were placed in a water-bath and imaged with a ultrasonographic scanner (Tringa linear). For ovulatory structures with a cavity, overall volume and volume of the cavity were calculated (volume = 4/3 × Pi × radius^3); the difference was the volume of luteal tissue. Digital images were made of intact structures and after they were opened by cutting with a surgical blade. Opened structures were then placed in 4% formaldehyde solution for at least 24 hours, trimmed, routinely embedded in paraffin, and sectioned. Histological slides were prepared and stained with haematoxylin-eosin, Azan blue and Gömöri's silver impregnation. Histological descriptions were based on the examination of slides with conventional bright-field light microscopy and specialised image-analysis software (CellD, Soft Imaging System GmbH). Various aspects of ovulatory and anovulatory structures were determined (Fig. 1). For ovulatory structures, the thickness of the connective tissue layer was measured and the number of large and small luteal cells, fibroblasts/fibrocytes and pycnotic cells were counted. The same calculations were made with 4 solid CL to compare data.

A mixed model analysis of variance (ANOVA) and Student's *t*-test was used for statistical analyses.

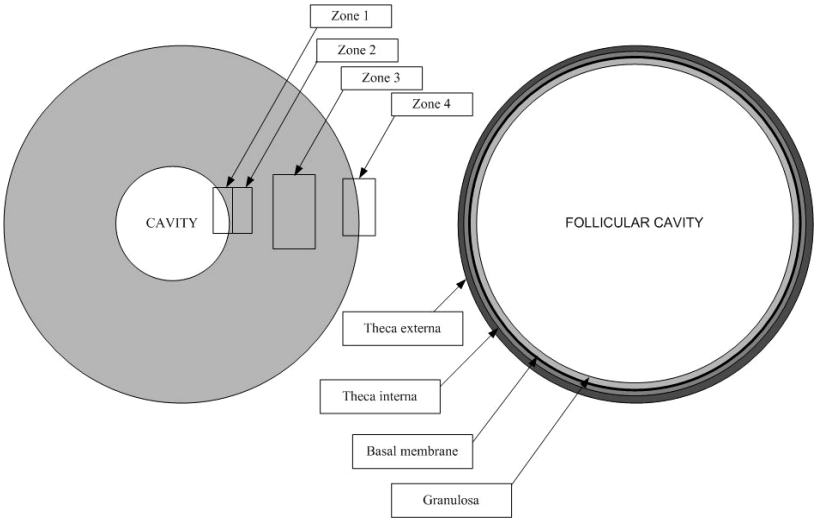

Fig. 1. Layers of ovarian structures studied by light microscopy (left: Groups A and B; right: Groups C, D and E)

Results

Incidence of fluid-filled ovarian phenomena in a dairy farm with a milk production higher than the Hungarian average in 2008–2011

Postpartum cows

During the study period, a total of 515 postpartum cows (treated according to the Provsynch regimen) were examined. The incidence of FFOP was 30.1%. The milk production and the body condition score did not have a demonstrable effect on the incidence of either FFOP or ovulatory or anovulatory forms.

Heifers examined after insemination

Pregnancy diagnosis of 796 heifers was carried out 28–50 days after insemination, when 484 were found to be pregnant (60.8%). FFOP was diagnosed in 92 open heifers (29.5%). In pregnant animals, non-homogeneous corpora lutea were regarded as FFOP. Among the 484 pregnant heifers, 4 heifers maintained their pregnancy with FFOP (0.8%), and one had a late embryonic loss (25%). In open heifers, the incidence of ovulatory FFOP was significantly higher than that of anovulatory FFOP (P=0.006).

Cows examined after insemination

Pregnancy diagnosis of 3226 cows (1399 pregnant – 43.4%) was carried out 28–50 days after insemination. The incidence of FFOP was 37.6%. In 30 cows, corpus luteum with fluid content maintained pregnancy (the rate of late embryonic loss was 16.7%). The incidence of FFOP was significantly higher in open cows than in open heifers (P=0.006). Anovulatory FFOP were diagnosed more often in open cows (P=0.01), while ovulatory FFOP were more frequent in open heifers (P=0.01). There was no significant difference in late embryonic losses between cows and heifers (P=0.686). No difference was found in the incidence of FFOP and in the distribution of ovulatory and anovulatory FFOP between postpartum and post-insemination cows.

Heifers and cows inseminated after different hormonal treatments

Of the heifers inseminated after a spontaneous oestrus (hSP group), 189 were diagnosed to be open and 56 had an FFOP (29.6%). In

heifers that were open after prostaglandin-induced oestrus (hPG group – n=118), the incidence of FFOP was 29.7%. The incidence of FFOP did not differ in the SP and PG groups.. There were significantly more anovulatory FFOP in the PG group (P=0.02) and more ovulatory FFOP in the SP Group (P=0.02).

The incidence of FFOP in open cows was 35.9% after spontaneous oestrus (cSP group), 42.2% after PG-induced oestrus (cPG group), 38.7% after Ovsynch-induced oestrus (cOVS) and 35.2% after Provsynch-induced oestrus (cPROV group). Hormonal treatments, milk production and body condition did not affect the incidence of FFOP. There were significantly more ovulatory forms in the tOVS group than in the tPG group (P=0.007). In the tPG group there were significantly more FFOP than in the tPORV group (P=0.032).

Comparing the data of open cows and heifers, ovulatory forms are more often found in the hSP group, while in the cSP Group anovulatory forms are more frequent (P=0.001). In the PG groups there were more FFOP in cows than in heifers (P=0.015).

Seasonal fertility differences in synchronised dairy cows: ultrasonic, metabolic and endocrine measures

In the summer period, 2 out of the 10 cows were excluded from the experiment because of metritis. None of the remaining cows re-conceived at the fixed-time AI. In the winter period, 1 cow out of 10 was excluded from the experiment due to metritis and 6 out of the remaining 9 cows became pregnant (66.7% pregnancy rate). In summer, FFOP were detected in 6 of 8 cows PI (75%), while in

winter in 2 cows out of 9 (22.2%). The mean BCS of the experimental cows at the time of AI was 2.73 in summer and 2.94 in winter, respectively.

In summer, the mean plasma NEFA concentration periodically increased above the upper limit of the normal range (<0.4 mmol/l) In winter, the mean plasma NEFA concentration exceeded the upper limit of the normal range only on days 56 and 63. In winter, a significant time-related decrease was found in plasma NEFA concentration post partum (P=0.0255). The plasma NEFA concentrations were significantly higher in summer than in winter (P<0.0001).

In summer, the mean plasma BHB concentrations showed a periodic fluctuation similar to that of the plasma NEFA concentrations, but they never exceeded the upper limit of the physiological range (<0.8 mmol/l). The mean plasma BHB concentration never exceeded the normal range in winter. Plasma BHB concentrations were significantly higher in summer than in winter (P<0.0005).

The mean plasma FRAP and the mean serum beta-carotene concentration remained within the normal range during both the summer and the winter study periods. The FRAP was higher in summer than in winter (P<0.0001), and after calving it showed a significant increase in summer (P<0.0001) and a significant decrease in winter (P<0.0001). The plasma T3 and T4 concentrations did not show any change between summer and winter. Plasma IGF-I concentrations were significantly higher in winter (P<0.0001).

The highest concentration was 4.3 µIU/ml (day 91) in summer and 6.36 µIU/ml (day 77) in winter (Fig. 6). No significant differences were found in mean plasma insulin concentrations between summer and winter, but significant increases were detected after calving in both periods ($P<0.0001$).

Endocrine and morphologic characteristics of fluid-filled ovarian phenomena

Biopsies and aspirations

I aspirated the fluid content of 15 FFOP from the ovaries of slaughtered cows and that of 11 FFOP *in vivo*. There were some extreme concentrations. Mean P4 concentration in aspirated fluids was the highest in Group A and the lowest in Groups D and E. Mean E2 concentration in the aspirated fluid was the highest in Group C and the lowest in Group A. P4 plasma concentration was the highest in Group B and the lowest in Group D. E2 plasma concentration was the highest in Group D and the lowest in Groups B and C. NEFA concentrations in aspirated fluids were highest in Group A, and in the plasma samples from Group B. There was a significant difference in P4 concentrations of aspirated fluids between Groups A and E ($P<0.0001$). Plasma P4 concentrations in Group B significantly differed from those of the other groups ($P<0.005$). A significant difference in the NEFA concentrations of aspirated fluids was found between Group A and Group E ($P<0.05$).

Morphological (ultrasonic, macro- and microscopic examinations)

Ovulatory phenomena [Groups A (n=6) and B (n=4)]

In Group A (a total of 6 CL with a cavity <1 cm and a wall thickness >1 cm), ultrasonographically there was a clear demarcation between the dark fluid-filled cavity and the homogeneous luteal tissue. Overall, the macroscopic appearance of Groups A and B (CL with a small or large cavity, respectively) was similar to that of a solid CL. When the structures were opened, the luteal tissue appeared similar to a solid CL, but all cavities were lined with shiny, grey tissue that was well capillarised (cavity wall with connective tissue, CWCT – Zones 1 and 2). Histologically, there were two major layers of the CWCT detected by Azan staining, as follows:

a. collagen fibres in the innermost CWCT and pycnotic cells or cellular debris among these fibres (Zone 1),
b. well-capillarised fibrous connective tissue with some normal luteal cells (Zone 2).

Under the CWCT, there was a layer of functional luteal cells (similar to a solid CL), albeit with more pycnotic and less mitotic cells (Zone 3). Reticular fibres, similar to a solid CL, were detected in sections stained with Gömöri's silver impregnation. Furthermore, in some structures from Groups A and B, there were connective tissue trabeculae, similar to those in a solid CL, present within the luteal tissue. At the margins of the structures (Zone 4), many fibroblasts and fibrocytes were surrounded by loosely organised connective tissue.

In Group B (CL with cavity >1 cm and wall thickness <1 cm), the lumen was surrounded by a thick connective tissue layer, with a thinner active luteal cell layer than in Group A. Overall, other structural characteristics were similar to those in Group A.

There was a greater proportion ($P<0.05$) of small luteal cells in Group B compared to a solid CL, whereas Group A was intermediate in this respect. There were also differences in the numbers of fibroblasts/fibrocytes and pycnotic cells ($P<0.05$ for each). Finally, connective tissue was thicker ($P<0.05$) in Group B than in Group A. The volume of luteal tissue was less ($P<0.05$) in Group B than in Group A, whereas a solid CL was intermediate in this regard.

Anovulatory phenomena

Group C (n=8)

A typical characteristic of anovulatory FFOP is the lack of ovulatory papilla. These were fluid-filled structures <2 cm in diameter, but with a thick, grey wall. Histologically, proceeding from the inside to the outside, the inner wall of these structures was lined by a thin layer (1–2 mm) of shiny, grey tissue. This tissue was well vascularised, but no yellow layer was detected. As the subsequent layer, in some of these structures, there were two or three layers of granulosa cells (these were sometimes missing). The basal membrane was recognisable, and below it there was an active theca interna (6 to 8

layers), but no luteinisation was detected. The theca externa, which contained well-organised connective tissue, was well vascularised.

Group D (n=5)

These structures were anovulatory, with a total diameter of >2 cm and a wall thickness of <3 mm. Ultrasonographically, they had a large fluid-filled cavity with a grey wall. Macroscopically, they were fluid-filled grey structures with a smooth surface. In some cases, a few yellow spots were present on the inner wall. Although the macroscopic and ultrasonic appearance of these phenomena was very similar, histologically there were two distinct types:

Type 1: The layers were clearly demarcated. The wall was usually 6–8 layers (sometimes less), although the granulosa layer was sometimes exfoliated. If the granulosa layer was intact, cells were active but non-luteinised. The basal membrane was usually visible, but sometimes it was broken. All theca layers were well-vascularised; active, hormone-secreting cells were present in the theca interna, but the number of luteinised cells was low. Theca layers usually consisted of many fibroblasts, capillaries and unorganised connective tissue (sometimes with finer filaments).

Type 2: The lumen was lined with a thick layer of collagen filaments, with only a few granulosa cells present.

Group E (n=4)

These were anovulatory structures, with a diameter of >2 cm and a wall thickness of >3 mm. Ultrasonographically, they were similar to Group D structures, but the wall was thicker, and occasionally dark-

grey extensions into the cavity were visible, perhaps representing partial detachment (protrusion) of the wall, attributable to the proliferation of luteal tissue. The outer surface was smooth, with a yellow or partly yellow wall, but no ovulation papilla. Histologically, there were parallel reticular fibres (connective tissue) around the inner wall of the cavity (detected by Gömöri's silver impregnation and Azan staining). These fibres penetrated into the luteinised cell layer, but less regularly than in Groups A or B (no trabeculae were detected). The theca interna and granulosa layers were not distinguishable; only luteinised cells (mainly large luteal cells) in approximately 15 cell layers were detected. No basal membrane was present, and the outside wall was formed by the theca externa, with fibroblasts and capillaries.

Discussion, conclusions

Incidence of fluid-filled ovarian phenomena in a dairy farm with a milk production higher than the Hungarian average in 2008–2011

In the literature, ovarian cysts are characterised as phenomena with a diameter bigger than 2 cm. In my studies, the incidence of FFOP was 37.6% in cows but their incidence did not differ post partum and post insemination (30.1% vs. 37.6%), although many authors suggest that ovarian cysts are more common post partum. There may be involution problems in the background (metritis, twin birth, etc.), but this effect can be excluded in our case because we had cows treated with the Provsynch protocol.

Open cows had more FFOP than open heifers (P=0.006), which is consistent with the data of the literature about the association between parity and the incidence of ovarian cysts. We could not detect an effect of body condition on the incidence of FFOP. Open heifers developed less anovulatory forms than ovulatory phenomena (9.3% vs. 20.2%).

No data could be found in the literature about the incidence of ovarian cysts in cows/heifers inseminated after different hormonal treatments. There was no difference in the incidence of FFOP between open heifers after spontaneous and PG-induced ovulation, but the percentage of ovulatory and anovulatory forms differed (P=0.02). Anovulatory forms developed more frequently in open heifers inseminated after PG treatment; this could occur because heifers treated with PG had not been detected as being in oestrus before („problem" animals).

Hormonal treatments were found to have a significant effect on the formation of FFOP in open cows. Open cows inseminated after Ovsynch developed significantly more anovulatory forms than open cows inseminated after PG (P=0.007). This was probably due to the absence of cyclic ovarian activity even after Ovsynch treatment and insemination. The proportion of FFOP was significantly higher in open cows after PG treatment than in open cows after Provsynch treatment (P=0.032). The lower incidence of FFOP in open cows after Provsynch treatment might be explained by the fact that the Provsynch protocol was used only in healthy animals, which did not have major postpartum problems in energy balance either.

In heifers remaining open after a spontaneous ovulation ovulatory forms were more common, while in cows anovulatory forms showed a higher incidence (P=0.001). A possible explanation can be that cyclic ovarian function can be influenced by fewer factors in heifers than in cows, and therefore FFOP can develop more frequently after ovulation.

Seasonal fertility differences in synchronised dairy cows: ultrasonic, metabolic and endocrine measures

During the warm summer period, the poor expression of oestrous signs, the rapid decrease of the pregnancy rate, and a pronounced increase of late embryonic losses (LEL) are typical events.

Summer heat stress affects the hypothalamic–hypophyseal–ovarian axis, the dominance of the selected follicle, the length of the follicular wave, the quality of the oocyte, and the energy balance. The intrauterine environment is also compromised in cows exposed to heat stress: blood flow to the uterus decreases and intrauterine temperature increases. These changes may inhibit embryonic development, increase early embryonic loss and reduce the proportion of successful inseminations. High ambient temperatures in summer may lead to changes in cell membrane properties which, in turn, can influence oocyte function and fertility.

In heat-stressed dairy cows, the reduction of appetite and, thus, of dry matter intake is a usual consequence. In our experiment, the mean BCS was 0.21 higher in winter than in summer, which should mean a slight decrease of body weight, resulting in lipid mobilisation

as indicated by increased plasma NEFA concentration. Decreasing body weight with low plasma insulin concentration and the release of stress-associated catecholamines due to heat exposure increase the degree of lipolysis and decrease the rate of re-esterification of free fatty acids in the adipose tissue. In parallel, the plasma concentrations of insulin, glucose and IGF-I decrease, whereas those of growth hormone and NEFA increase. In the present study, average plasma insulin and IGF-I concentrations were significantly higher in winter than in summer ($P<0.01$), supporting the idea of less lipid mobilisation in winter.

Mobilised lipids result in a subsequent increase of plasma NEFA and BHB concentrations. The significantly higher serum NEFA concentration found in summer was probably caused by the reduced appetite (due to the high ambient temperature, which may have resulted in a lower energy intake, as indicated by the higher amount of TMR remaining in the feed bunk), expressed in a lower BCS. Mean plasma BHB concentrations were higher in summer than in winter, but they never exceeded the physiological range, which is indicative of energy mobilisation but not ketosis. Statistical analysis demonstrated a significant effect of season on plasma NEFA, BHB and IGF-I concentrations ($P<0.001$). NEFA have a negative effect on granulosa and theca cells *in vitro* and probably also *in vivo*.

Several authors have stated that the appearance of irregular luteal forms (ILFs) may be due to many circumstances but the most common factors are (1) inflammation (because of the role of cytokines), (2) metabolic abnormalities, and (3) endotoxins and/or

stress. Since in these experiments only healthy animals were treated with the Provsynch regimen, the general effect of metritis and/or inflammation can be excluded. The experimental design did not differ between summer and winter, so ambient temperature was the only environmental factor that could influence the formation of ILFs. The incidence rate of ILFs was 75% in summer and 22.2% in winter. Statistical analysis supports the association between the formation of ILFs and higher plasma NEFA and lower plasma IGF-I concentration. Based on our findings, it seems that the appearance of ILFs may be caused by the effects exerted by NEFA on the theca and granulosa cells of the follicle.

In conclusion, statistical analysis supports the hypothesis that increased plasma NEFA and BHB and decreased plasma IGF-I concentrations may result in reduced fertility in summer. These changes may be associated with the more frequent appearance of ILFs and probably had a negative effect on ovarian function and/or oocyte quality.

Endocrine and morphological characteristics of fluid-filled ovarian phenomena

Aspirations

The follicular fluid has many biochemical components, which are essential for ovarian function (steroid production, folliculogenesis and ovulation). Based on our findings, P4 and E2 concentrations (in the plasma and in aspirated fluids) did not show clear correlations

with the morphological characteristics in all cases (this is described in detail in the evaluation of the morphological findings).

NEFA concentrations in fluids aspirated from ovulatory forms were higher than in those obtained from anovulatory forms. Due to the higher NEFA concentrations in the aspirated fluids and also in the plasma, accumulation of NEFA in the forms could have a role in their formation.

Morphological (ultrasonic, macro- and microscopic examinations)

'Ovarian cyst' is the term most commonly used in the literature to designate FFOP. Diagnosis is mostly based on diameter and wall thickness, and only few authors underline the importance of origin (ovulatory and anovulatory forms). In the present study, ovarian structures were defined as either ovulatory (solid CL; Groups A and B) or anovulatory (Groups C, D and E) on the basis of the presence or absence of an ovulation papilla.

Ovulatory forms

It was noteworthy that the proportion of small luteal cells was significantly greater in Group B than in solid CL (Group A was intermediate in this respect). Since all cavities disappeared over time, we inferred that, on average, large cavities would be the youngest luteal structures, a solid CL would be the oldest, and a small cavity would be intermediate. The observation that Group B structures had the largest proportion of small luteal cells was consistent with the prediction that they were the youngest luteal

structures, since it is well established that some small luteal cells become large luteal cells over time.

Connective tissue thickness was significantly less in Group A than in Group B. Connective tissue forms after differentiation of granulation tissue (formation of collagen and elastic fibres) from the adventitia of blood vessels. Perhaps the connective tissue lining the cavity was due to post-ovulation proliferation and response to tissue damage due to hydrostatic pressure of the liquid. In that regard, Group B structures, with their larger cavities, would be expected to have more damage and consequently more connective tissue. Furthermore, between the fibres of connective tissue layers, there were some damaged luteal cells.

The high P4 concentrations of aspirated fluids from Groups A and B indicate similarity to the homogeneous CL. Plasma P4 concentrations were the highest in Group B ($P<0.05$). Granulosa cells even show enhanced and more efficient steroidogenesis during apoptosis, due to clustering of the steroidogenic organelles, which could explain the high P4 concentrations. High NEFA concentrations were measured in aspirated fluids of Groups A and B, which could affect the formation of these structures. No data could be found in the literature on the hormone and NEFA concentrations of fluids aspirated from the ovulatory forms.

Group A structures had an ultrasonographic morphology similar to that of a solid CL, except that they also had a non-echodense cavity <1 cm diameter with a limited layer of connective tissue surrounding the cavity, and a wall at least 10 mm thick. Therefore, we suggest

that these structures should be designated as *corpus luteum with a cavity*. In contrast, we suggest that Group B structures, with a cavity >1 cm, a well-marked CWCT and a total wall thickness <10 mm, should be designated as *cystic corpus luteum*, consistently with a similar, previous assertion.

Anovulatory forms

By definition, anovulatory structures are follicles that failed to ovulate. Although the exact pathogenesis of failure to ovulate is unknown, the general consensus is that a hypothalamic-pituitary dysfunction is involved. Classification of these structures is usually based on their size, persistence, blood progesterone concentrations, and absence of a concurrent CL.

The ultrasonographic and histological appearances of phenomena in Group C (diameter <2 cm, wall 1–3 mm, and no ovulatory papilla) were somewhat similar to those of a normal follicle. However, their thicker wall consisted mainly of connective tissue, and only a few (or no) granulosa cell layers and no luteal cells. Based on these attributes, these structures were consistent with *anovulatory/-persistent follicles* reported previously.

In aspirated fluids of Group C, E2 concentrations were high, which can cause oestrous signs in live animals. NEFA concentrations were usually lower than in ovulatory forms, but the reason is not clear because of the unknown age of the structures.

Phenomena in Group D (diameter >2 cm, wall <3 mm, no ovulatory papilla) had a similar macroscopic structure as follicles; however,

differences included size, wall thickness and the presence of yellow spots on the inner side of the wall. Histologically, they either had layers similar to those in a follicle (with some luteinised cells in the theca interna layer) or their wall consisted only of some desquamated granulosa cells and thick connective tissue (Type 1 and Type 2, respectively). Perhaps the presence of luteal tissue accounts for regression of some of these structures following a single prostaglandin treatment, as we have reported recently.

Hormone production of structures in Group B is characterised by high concentrations of E2, reaching the level normally found during oestrus. According to that, cows could be inseminated, but conception did not take place (no ovulation). Based on the high concentrations of NEFA measured in the plasma and in aspirated fluids, these structures can cumulate NEFA, which can play a role in their formation. Although these structures are usually termed as 'follicular cysts', to make it clear that not only this type of cyst is formed from anovulatory follicles and based on the substantial presence of connective tissue fibres, the term '*follicle-fibrous cysts'* is suggested.

Large anovulatory cysts with almost continuous luteinisation in their wall (visible by ultrasound and also macroscopically) had a completely different histological structure than did other anovulatory cysts (Group E: diameter >2 cm, wall >3 mm, no ovulation papilla). Their microscopic appearance was more similar to that of a CL; collagen fibres around the cystic cavity penetrated into the luteinised cell layer, but in a less regular arrangement (trabecules were not

present) than in ovulatory structures. Luteinised cells were primarily identified as large luteal cells, but a few small luteal cells were also present.

Based on the E2 and P4 concentrations measured in the aspirated fluid and plasma, the *in vivo* presence of these structures could cause mixed clinical signs: oestrous signs can develop, but the P4 production of the structures can block the ovarian cycle. NEFA concentrations in aspirated fluids were significantly lower in Group E than in Group A ($P<0.005$), and the plasma NEFA concentrations were within the physiological range.

Based on their anovulatory status and substantial luteinised cell content, we suggest that the term '*follicle-luteinised cyst*' is more appropriate for designating these structures than the name 'luteal cyst'.

The high prevalence of fluid-filled structures in bovine ovaries and the inconsistency in terminology provide ample justification for further investigation. Macroscopic, ultrasonographic and histological features should help in categorising these structures. The presence (and thickness) of the connective tissue layer around a central cavity may be indicative of the persistence of the structure. Differences in cell distribution (small luteal cells, fibroblasts/fibrocytes, pycnotic cells), connective tissue thickness and luteal tissue volume were consistent with the differentiation of CL with cavity and cystic CL. Due to the existence of several transient forms, the differentiation of non-ovulatory phenomena is more difficult, but 1) the absence of an ovulation papilla, 2) overall diameter and 3) wall thickness could

provide enough information for making a correct ultrasonographic diagnosis of these structures. Based on the results of the present study, the author suggests that a slight modification of nomenclature would be appropriate (Fig. 2).

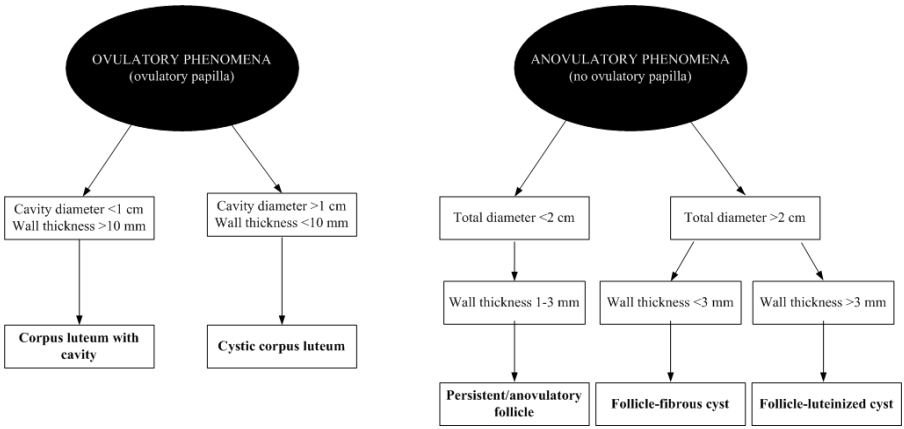

Fig. 2. Suggested nomenclature for fluid-filled ovarian structures

New scientific results

1. There is no significant difference in the incidence of FFOP between cows open post partum and those remaining open after insemination. Milk production, body condition and hormonal treatments before insemination do not have a direct effect on the formation of FFOP.

2. FFOP could be detected more frequently in open cows than in open heifers examined after insemination.

3. Elevated plasma concentrations of NEFA may have a role in the formation of FFOP, because higher numbers of FFOP could be detected in animals with high plasma NEFA concentrations and when the NEFA content of aspirated fluids was also high. High plasma NEFA (and BHB) and low IGF-I concentrations are associated with low fertility in summer.

4. Ovulatory FFOP differ from the homogeneous CL in the presence of the fluid-filled cavity and in cell content. The thick connective tissue layer of the cavity wall indicates a pathological process; this is why ovulatory FFOP can not always be considered physiological.

5. High E2 production of anovulatory FFOP (detected in plasma and aspirated fluids) could cause oestrus-like signs in live animals, but sometimes P4 production can also be elevated (luteinised cysts). The incidence of fertility problems (unsuccessful inseminations, blocking of the ovarian cycle) increases in both cases.

6. I have elaborated a proposal for modifying the nomenclature of FFOP (Fig. 2).

Publications related to the topics of the present thesis

a, Full-text papers published in peer-reviewed journals in English

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