Large ovary syndrome in cattle: a retrospective study

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Abstract

Similar to horses, large ovary syndrome in cattle (LOS) is characterized by an excessive size and deformity of 1 or both ovaries. Most often, a bovine practitioner refers these cases to a theriogenologist to confirm diagnosis and provide treatment. Objectives were to review the process of diagnosis and to assess factors impacting fertility after removal of the large ovary in cows with LOS. We present 20 LOS cases referred to the veterinary teaching hospital of the University of Montreal (CHUV). Data were collected on calving history, cyclicity, previous treatments, results of general and reproductive examinations including transrectal palpation and ultrasonographic evaluation, and histopathological results after ovary removal. Twelve LOS cases (60%) were identified as granulosa-theca cell tumors (GCT), 4 (20%) as ovarian abscesses (OA), and 4 (20%) as ovarian hematomas (OH). Anestrus was the most noticeable clinical sign reported by owners. On average, the ovarian volume (height x length x width) was 1,945, 1,187, and 524.2 cm^3 for GCT, OA, and OH respectively. Concomitant conditions, such as endometritis and urovagina, had negative impacts on fertility. From these data, we concluded that cows presented with LOS postpartum warrant a complete reproductive examination. Although 95% of the cows demonstrated a good outcome after surgical removal of the large ovary, untreated concomitant reproductive disorders resulted in culling.

Keywords: Ovarian anomaly, infertility, diagnosis, prognosis

Introduction

Large ovary syndrome (LOS) can manifest itself through a variety of clinical signs.[1] Various ovarian pathologies causing the LOS have been described with the following overall prevalence of reproductive disorders in cattle: ovarian cysts (OC) 6 - 19%,[2] ovarian hematoma (OH) < 1.5%, ovarian abscess (OA) < 1.0%, oophoritis and perioophoritis (OO) < 1.0%,[3] ovarian neoplasms such as granulosa-theca cell tumors (GCT) 0.5%,[4] ovarian adhesions (OAD) 1.5%, developmental anomaly 0.2%, and periovarian tumor (POT) < 0.5%.[3]

Most commonly diagnosed ovarian abnormality by a bovine practitioner is the ovarian follicular cyst (FC). A preliminary diagnosis of the condition is usually based on a transrectal palpation of the ovaries and a history of anestrus.[5] Only 2% of cows with OC present frequent and irregular signs of estrus with edematous swelling of the vulva and abundant clear mucus discharge, whereas 74% of cows present anestrus as the main sign.[6] Cows with an ovarian cyst are most often identified at first reproductive system examination in the postpartum period or at the first examination for pregnancy. Generally, diagnosis of follicular cysts by transrectal examination is confirmed by ultrasonography. FC appears as a large fluctuating anechoic fluid-filled structure > 25 mm in diameter with a very thin outer wall of < 3 mm and in the absence of a corpus luteum.[7] Solitary cysts are more common than multiple cysts. In chronic cases, sinking of sacral ligaments, upward displacement of the coccygeal vertebrae, and thickening of the neck and brisket may be observed.[8] Cows diagnosed with OC have a dysfunctional hypothalamic-pituitary-ovarian feedback loop resulting in a basal peripheral progesterone (P_4) concentrations and increased estradiol concentrations especially at the early stages of cystic development. Aberrant LH pulse frequency and amplitude translate into an inadequate LH surge necessary for ovulation to occur. Studies suggest that in early postpartum, follicular cyst development is associated with disrupted insulin signalling pathway due to a negative energy status of the newly lactating cow.[9] Various hormonal treatments are routinely used, either GnRH (gonadotropin-releasing hormone) or hCG (human chorionic gonadotrophin) alone or an additional prostaglandin treatment ~ 9 - 14 days later.[10] A synchronization protocol (Ovsynch) is frequently used to treat cows with OC. If treatment remains unsuccessful and the ovarian size continues to increase, a more detailed examination of the reproductive tract should be performed to rule out other ovarian anomalies.[11]

In contrast, OH and OA are uncommon conditions with unclear and ambiguous etiology and less defined clinical manifestations.[12] Because the animal may still cycle normally, the finding is often unexpected and difficult to diagnose by most veterinarians and supplementary diagnostic procedures should be carried out by a theriogenologist and a histopathologist to make a definitive diagnosis. Limited information is available for OH in the literature. In cases of OA, an ascending infection of the uterus travelling via oviduct to the ovary is suspected.

Some earlier reports considered enucleation of the corpus luteum as a possible cause and more recent studies described follicular aspiration, a technique which has gained increased applications in the last decades, as a major culprit. Inflammation of the ovary (oophoritis), including surrounding structures (perioophoritis) are rare conditions in farm animals and may be observed in connection with uterine infection and peritonitis.[13] Paraovarian cysts occur quite rarely compared to horses and are usually not included in the list of a differential diagnosis in cattle LOS cases. Paraovarian cysts are cystic structures that occur in the broad ligament adjacent to the ovaries and can be mistaken for follicular cysts.[14] Ultrasonography alone may not be diagnostic for this type of cyst and meticulous transrectal palpation generally provides a better sense of cyst location, type, and mobility.

Ovarian tumors are rare in cattle and classified into 3 categories: surface epithelial tumors (e.g. cystadenomas and papillomas), sex cord-gonadal stromal tumors (e.g. granulosa-theca cell tumors and fibromas), and germ cell tumors (e.g. teratomas and dysgerminomas).[15] Sex cord-gonadal stromal tumors are the most common ovarian neoplasm in cattle.[16] These are a heterogeneous group of benign and malignant tumors, including tumors composed of cells that produce ovarian hormones like granulosa cells and granulosa-theca cell tumors.[17] Sex cord-gonadal stromal tumors may produce steroid hormones that result in either nymphomania, mainly at the early stage of the condition followed by anestrus or virilism in long-standing cases.[18] These tumors contain mixed areas of specialized gonadal stroma: cortical and medullary stromal cells, granulosa cells (GCs), and follicular theca internal cells. Most ovarian tumors are diagnosed as a mass approximately the size of a fist inside the abdominal cavity; however, they can reach > 30 cm in diameter in severe cases.[19] In sex cord-gonadal stromal tumors, the contralateral ovary is typically regressed and without follicular activity due to inhibin and AMH production by the ovarian granulosa cell tumor.[18,20] Objectives were to review the process of diagnosis and to assess factors impacting fertility after removal of the large ovary.

Materials and methods

Terms for search and file data used for analysis

We identified animal admissions with a 'large ovary' as the main cause for referral in VetView, (Veterinary Hospital & Laboratory Management System, Athens, GA) and reviewed bovine hospital logbook and theriogenology service of the faculty of veterinary medicine at the University of Montreal (CHUV) for the years 2012 - 2020. All procedures were approved (protocol number IA120633) by Comité d'éthique de l'utilisation des animaux of the University of Montreal.

Individual files were thoroughly analyzed and the data compiled. To determine short- and long-term prognosis, we collected follow-up information by phone interviews with owners and referring veterinarians. A positive short-term outcome was defined as a return to normal milk production within 1 week after hospital discharge. A positive long-term outcome was defined as the cow becoming pregnant within 2 months of hospitalization discharge. Initial diagnosis of LOS was made by the referring veterinarian in a routine reproductive visit of the herd. Analyzed data included reason for referral, animal description (age, days in milk, and duration of clinical signs), previous treatments on the farm, and case history.

Description of the population

CHUV receives referrals from herds in the provinces of Quebec and Ontario. Herds represent mainly Holstein dairy cows, with an average of ~ 80 cows/farm in Quebec and 100 cows per farm in Ontario.

Clinical examination at CHUV

A complete physical examination (temperature, heart rate, and respiratory rate) was performed on all cows upon admission. Transrectal palpation was performed to determine uterine size and to identify corpus luteum (≥ 20 mm diameter), follicles (≥ 10 mm diameter), and cysts. Transrectal ultrasonography was performed with a 7.5 MHz linear probe (Ibex, E.I. Medical Imaging, Loveland, CO). Vulva was cleaned, dried, and wiped with an alcohol gauze and a disposable vaginoscope moistened with a minimal amount of sterile lubricant was inserted into the vagina. With a light source, the vaginal vault and the cervix were examined visually for discharge that was categorized using a 4-point classification system: 0 = no discharge, 1 = clear and translucent mucus, 2 = cloudy mucus with or without flecks of pus (< 50% pus), 3 = mucopurulent discharge (> 50% pus), and 4 = purulent discharge.[21] Clinical endometritis was defined as a purulent vaginal discharge (score of > 2) after 21 days postpartum.[22] Cytological endometritis or subclinical endometritis was defined based on > 5% neutrophils on endometrial cytology without vaginal discharge after 21 days postpartum.[23] Cervix was classified as grade 0 (normal) without abnormality; grade 1 (normal) with the second cervical fold swollen without redness and prolapsing through the first ring, and grade 2 with the second fold being swollen, red and prolapsing through the first ring without purulent vaginal discharge.[23] Clinical cervicitis was defined as a cervical grade of 2.

Sampling for uterine cytology and bacteriology

Cytobrush technique was used to collect samples of mucosal cells from the uterus to diagnose a possible subclinical endometritis. Briefly, cytobrush samples were collected using a modified Cassou gun. Double-protected Cassou containing the cytobrush was inserted in the vagina and placed in front of the external cervical ring, the double-pipette perforated and the Cassou gun containing the cytobrush was passed through the cervix. Once the uterine body was reached, the cytobrush was exposed and rolled over the mucosa to collect cells and then withdrawn into the Cassou gun

before removing it back out through the cervix and vagina in the double-pipette. Cytobrush was then rolled on a sterile glass slide, air-dried, and stained. Smears were examined under the microscope at 400 x magnification, and a total of 200 cells were counted and characterized. Percentage of polymorphonuclear neutrophils (PMNs) was determined for each cow.

After rolling the cytobrush on a sterile slide, it was cut and packaged in a sterile plastic tube and transported to the diagnostic bacteriology laboratory of the Faculty of the Veterinary Medicine. In the laboratory, bacteriological samples were cultured for aerobic and anaerobic bacteria using standard methods for bacteriological testing (API System: bioMerieux, Marcy-Etoile, France). For microbiological analysis, the brushes were plated onto sheep blood agar with a sterile disposable plastic loop (soy agar with 5% sheep blood, Becton Dickinson, Franklin Lakes, NJ). Plates were incubated for 48 hours at 35°C under aerobic conditions and then examined. When growth was observed, colony types were identified based on morphology, pigmentation, and hemolytic patterns. Organisms were further subcultured and identified using biochemical profiles and other standard methods. Plates containing > 1 colony-forming units were considered positive for bacterial growth.[24]

Blood sampling and analysis

All laboratory tests were performed by the CHUV diagnostic laboratory. Blood was collected via venipuncture of the coccygeal vein to assess P_4 and a complete blood panel was performed.

Hemiovariectomy via flank laparotomy

Feed was withheld for 36 hours prior to surgery. Antibiotic (procaine penicillin G 22,000 IU/kg every 24 hours) was given preoperatively to all cows and for at least 72 hours after surgery. Surgeries were performed in standing position by an ipsilateral caudal flank approach under a proximal paravertebral anesthesia of the last thoracic and the first 2 lumbar spinal nerves or by a line block at the incision line with 2% lidocaine (Vetoquinol N.-A. Inc., Lavaltrie, QC, Canada). After aseptically preparing the surgical site, a vertical incision below and cranial to the tuber coxae was performed. External oblique abdominal muscles were incised and the internal abdominal and transversus abdominis muscles were bluntly dissected before incising the peritoneum. Ovarian pedicle was double ligated with 2 braided glycolide/lactide copolymer (Polysorb, Covidien, Mansfield, MA) before sharp transection. In some cases, the transection was performed using a ligature (Covidien). Peritoneum and transversus abdominis muscle, and the internal and external oblique abdominal muscles, were sutured together using 2 braided glycolide/lactide copolymer (Polysorb, Covidien) in a simple continuous pattern. Skin was closed with 2 polycaprolactam (Supramid, Serag-Wiessner, Naila, Germany) in a Ford interlocking pattern. Hematocrit was measured immediately prior to and 24 hours after surgery to assess the risk of internal bleeding. Owners were instructed to remove skin sutures ~ 14 days after surgery and 3 weeks before attempting artificial insemination.

Macroscopic and histopathological examinations

Representative samples of the ovary were preserved in 10% formalin for 24 hours before being dehydrated in alcohol and embedded in paraffin. Thin tissue sections (4 µm) were made and placed onto slides for hematoxylin and eosin staining. After a general assessment, the pathologist randomly chose 5 areas under 400 x magnification for each slide before undertaking a complete evaluation.

Results

Animals

A total of 24 cases of LOS were identified. Four cases had to be excluded from the study because of incomplete medical history. Of these 20 cases, 12 were diagnosed with GCT, 4 with OA, and 4 with OH (Table). All animals were Holstein cattle with an average age of 3.5 years (6 months to 4.5 years). One animal was a heifer and the others were milking cows ranging from 90 to 240 days in milk (DIM), with a median DIM of 145 days. In total, 75% (3/4) of OH cases were considered to be in anestrus and 25% (1/4) were repeat breeder cases. In OA cases, 50% of animals were in anestrus and repeat breeders, respectively. However, in ovarian GCT, 42% (5/12) were in anestrus, 50% (6/12) were presented as a repeat breeder and 8% (1/12) were nymphomanic at admission. Approximately 95% of cows had a normal calving history. California mastitis test (CMT) and the leukosis test were negative for all cases, with the exception of 1 leukosis positive cow in the OA group. None of the cows had signs of systemic disease, such as fever, anorexia, nor lethargy. All animals were alert upon admission and in good body condition (3.0 - 3.75). Heart and respiratory rates were within normal limits. The median length of days in milk (DIM) for the OH, OA, and GCT groups were similar: 4 months (3 - 6 months), 4.5 (4 - 5 months) and 4 months (3 - 8 months), respectively.

Reproductive system examination

Percentage of cows with an enlarged ovary on the left side was 50, 50, and 75, for the OH, OA, and GCT groups, respectively (Table). In all cases, the contralateral ovary was static without active structures. The uterus was < 3 cm diameter and symmetrical without adhesions, via transrectal ultrasonography. Vaginoscopy examination via a speculum revealed a normal vagina with the exception of 1 OH cow with urovagina and pneumovagina, 1 OA cow with cervicitis[25] and 2 GCT cows with an urovagina and a pneumovagina, respectively (Table). Average size of the enlarged ovary for the OH, OA, and GCT groups were 10 x 5 x 4 cm, 8 x 10 x 4 cm, and 12 x 9 x 7 cm, respectively. Structures such as corpus luteum or dominant follicles could not be palpated on the contralateral ovary in cows with GCT and no abnormal lymph nodes were identified.

Table. Ovarian anomalies associated with large ovary syndrome and their characteristics

Ovarian anomaly	Ovarian hematoma	Ovarian abscess	Granulosa cell tumor
Number of cases	4	4	12
Age (years)	4.3	2-4	0.5 - 4.5
Reproductive signs	3 anestrus, 1 repeat breeder (urovagina)	2 anestrus, 2 repeat breeders	5 anestrus, 6 repeat breeders, 1 nymphomaniac
Calving history	Normal, 3 - 6 months postpartum	3 normal, 1 dystocia, 4 - 5 months postpartum	Normal, 3 - 8 months postpartum
CMT	Normal	Normal	Normal
Leukosis test	Negative	Positive for 1 case	Negative
Transrectal examination	4 large left ovary (LO), well involuted uterus	2 large LO, 2 large right ovary (RO)	9 large LO and 3 large RO
Vaginal examination	3 normal, 1 pneumovagina and urovagina with no discharge	3 normal, 1 cervicitis with no discharge	10 normal, 1 urovagina and 1 pneumovagina
Transrectal ovarian echography	2 RO: static, LO: 10 x 5 x 4 cm 2 LO: static, RO: 9 x 5 x 4 cm	2 RO: static, LO: 7 x 10 x 4 cm 2 LO: static, RO: 8 x 11 x 3 cm	9 RO: static, LO: 12 x 8 x 5 cm 3 LO: static, RO: 10 x 7 x 5 cm
Hormonal assay (range)	P_4: 1.4 - 1.7 ng/ml	P_4: 1.3 - 1.8 ng/ml	P_4: 0.2 - 3.4 ng/ml
Biopsy	A large proportion of the ovarian parenchyma is occupied by large coalescences of hemorrhage and necrosis	No ovarian structure, strongly infiltrated fibrous tissue of lymphocytes, plasma cells, macrophages and a smaller number of neutrophils	Micro- and macro-follicular structures arranged in anarchic form in an abundant cellular stroma; anisocytosis and anisokaryosis are moderate
Bacterial culture	No ovarian sample taken	*Trueperella pyogenes* (4 cases)	No ovarian sample taken

Echogenicity of the OA varied from anechoic to hyperechoic and homogenous to heterogenous, and was surrounded or not by a capsule (thick wall). More circumscribed and mature was the core of the abscess, the more hyperechoic it became and more evident was the surrounding anechoic or hypoechoic halo and acoustic enhancements (Figure 1C). In the case of a homogenous anechoic content, the thickness of the wall may be a distinctive characteristic compared to OH (Figure 1B) and GCT (Figure 1D). Abscesses are occasionally partitioned by echogenic bands, making differentiation from GCT difficult. Presence of adhesions around the ovary (oviduct, fimbriae, and surrounding tissues) on transrectal palpation should be expected in cases of OA because of localized inflammation. Ultrasonographically, GCT had multiple hypoechoic and hyperechoic regions and, unlike normal follicles that are distributed in the periphery of the ovary, hypoechoic fluid-like structures of varying sizes were distributed throughout the whole ovary and presented angular borders. Some GCT ovaries had a honeycomb appearance and others only few fluid structures and a more echogenic ovarian texture.

Surgical findings

Except for the OA cases, no adhesions were observed on the reproductive tract. In the 4 cases of OA, minor adhesions between the ipsilateral uterine horn, the rectum, and the abdominal wall were noticed. However, there was several pus-filled structures in the ovarian parenchyma that each one was encapsulated by a thick fibrotic capsule and in some case some pus was oozing during excision and exteriorization of the affected ovary (Figure 2C). In gross examination, making a differential diagnosis between OH and GCTs is not always feasible since in both cases the ovary looked like larger, more firm, lobulated and more spherical. It was only after dissection of the ovary following excision that this differentiation could be assessed. In OH, the presence of a large, single, blood-filled cavity in the center of the ovary that was surrounded by a thick capsule and also embedded by the ovarian echotexture (Figure 2B) was distinguishable from that of GCTs in which the surface of the ovary resembled more lobulated and more firm (Figure 2D). After removal and dissection of the ovary in OHs fresh blood gushed out of the cavity. However, in GCTs, depending on the cellularity type, the fluid inside the cavities had lower quantity although it was less sanguine. No evidence of uterine abnormalities was observed in any of the above cases. In most cases, the large ovary could not be exteriorized before excision complicating the hemostasis of large vessels of the mesovarium.

Laboratory analyses

Hematology and biochemistry analyses revealed mild changes in all groups, with a mild neutrophilia and lymphopenia. Mean and range of serum P_4 concentrations were 1.5 ng/ml (1.4 -1.7) for OH, 1.6 ng/ml (1.3 - 1.8) for OA, and 1.2 ng/ml (0.2 - 3.4) for GCT respectively. *Trueperella pyogenes* was isolated from the abscess of all cows with OA (no bacterial culture sample was

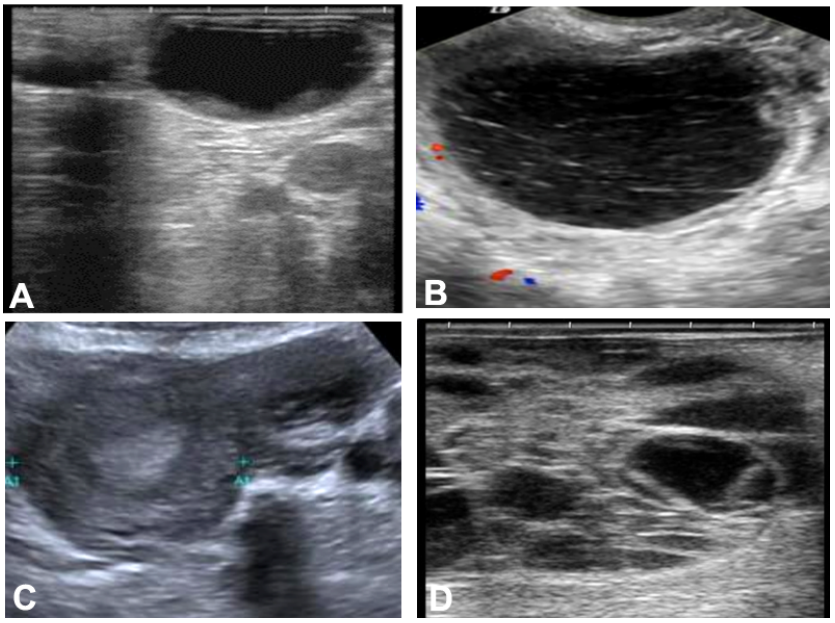

Figure 1. Differential diagnosis of left ovary based on transrectal ultrasonography. A. follicular cyst; usually smaller than other anomalies and hypoechoic, B. ovarian hematoma; hemorrhagic cavity filled with heterogenous echogenic liquid containing fibrin materials, C. ovarian abscess; multiple hyperechoic structures within the ovarian context, and D. granulosa-theca cell tumor; very large multi-compartmental and lobular structures throughout the ovary.

Figure 2. Differential diagnosis of left ovary based on gross examination of 4 large ovaries after ovariectomy. A. follicular cyst; large fluid-filled structure at the surface of the ovary, B; ovarian hematoma; large red and hemorrhagic structure at the surface of the ovary, C. ovarian abscess; multiple encapsulated and suppurative structures localized to different parts of the ovarian tissue, and D. granulosa-theca cell tumor; multilobular and multifollicular shape on the ovarian surface.

taken from OH and GCT groups). Endometrial cytology results were within the normal range (< 5% neutrophils).

Histologically, a large proportion of OH cases had coalescence of hemorrhage and necrosis infiltrated by inflammatory cells. In OA and OH cases, there was scant ovarian tissue. In OA cases, the ovarian stroma was replaced by collagenous tissue infiltrated by lymphocytes, plasma cells, and neutrophils. In GCT cases, the ovaries presented cell density of round or polygonal cells, often organized in superimposed and disorganized cellular layers forming a multitude of irregular lobules of variable sizes, and infiltrated with inflammatory cells. These irregular lobules resembled follicles with varying degrees of anisocytosis and anisokaryosis. In some cases, the cell infiltration was not capsulated. The round cells (granulosa cells) had formed micro- and macro-follicular structures arranged in anarchic form in an

abundant cellular stroma, showing anisocytosis, anisokaryosis, multiple nucleoli. Rate of differentiation was high, and no necrosis was observed in histologic sections, an indication of benign tumors. Mitotic index for 11 of 12 samples was below 10 mitoses per 10 high-power fields. One sample had a mitotic index above 19 mitoses per 10 high-power fields.

Short- and long-term outcomes

All cows had a good short-term prognosis, returning to normal production within 1 week after being released from the hospital. As expected, the priority of farmers was to have these cows return to normal fertility. All cows became pregnant within 2 months after being released from the hospital (19/20 pregnant), with the exception of 1 cow with urovagina and pneumovagina (OH). In this particular cow, urovagina and pneumovagina were not treated during hospitalization.

Discussion

Based on our data, LOS occurred in 0.5% of dairy cow referrals to the CHUV (24 of 4,500 total cases). In total, 60% of these cases of LOS were associated with GCT, for an incidence rate of 0.03% per year. A similar incidence of ovarian tumors was obtained with abattoir-derived material (0.7%, 11/1,480 cases).[26] Although ovarian tumors have been reported as more prevalent in animals over the age of 11 years, animals of all ages can be affected.[27] The dairy industry is currently culling cows on average in the third lactation and only 40% of culled cows have 3 lactations or more (Canadian Dairy Information Center, CDIC). Mean age of cows in the present study was 3 years (median: 3.5 years) and the mean number of lactations was 2. Of the 12 cows with GCT, there was only 1 heifer, 6-months old, without mammary gland development.

None of the cows was pregnant at the time of the LOS diagnosis, although GCT has been reported for various parities, pregnant and nonpregnant cows.[28] Such reported cases in pregnant cows reflected most likely an early form of the disease in which the tumor was small and not producing sufficient quantities of hormones to interfere with the normal reproductive cycle of the affected cow. In the present study, GCT occurred on the left ovary in 75% of cases (9 of 12); however, it can occur on either side.[29]

Incidence of OA and OH was < 0.1% (8/4,500). Surveys of reproductive tract examinations of cows at abattoirs had very low incidences: 0 cases in a study of 8071 cows and a single case in a study of 1,300 cows.[30] Given the substantial increase in the use of new biotechnology such as follicular aspiration for in vitro fertilization or follicular wave synchronization, one would have expected a greater incidence of these conditions.[31]

Most of the cases in the present study were diagnosed during a routine reproductive health visit, at a median DIM of 145 days that represents a time in lactation when > 50% of cows in the herd should have been diagnosed pregnant.[32] Late diagnosis of LOS may be due to the use of systematic synchronization protocols as reproductive management tool associated with the absence of a complete reproductive examination in early postpartum (30 - 40 DIM). No clear conclusion could be drawn from the present data. A late presumptive diagnosis of LOS can have negative consequences for the affected cow and the herd because finding the condition in late lactation increases the risk of culling. An early diagnosis would increase the chance of a quicker return to production and pregnancy. In addition, an earlier diagnosis offers an easier approach of hemiovariectomy via colpotomy, because of the smaller size of the affected ovary in early development of the condition. However, a smaller ovary would complicate the differential diagnosis. Success of surgical approach for removal of a large ovary depends on the size and the location of the ovary. In order to safely remove the enlarged ovary in the case of OH and GCT by colpotomy, the ovary should be < 8 - 10 cm in diameter (i.e. can fit in the palm of the hand). Nonetheless, removal of large GCT, OH, and OA via colpotomy can increase the risk of hemorrhage (OH, GCT) and peritonitis (OA).[33] However, colpotomy can potentially be performed at the farm with less expense and sanitary risk.

Once the primary complaint, such as anestrus, or less frequently, nymphomania is presented to the veterinary practitioner, the most important step of the initial examination to be performed is transrectal palpation and ultrasonographic examination of the ovaries. In the case of LOS, a very systematic approach to assess the form, size, localization, consistency, mobility, and ultrasonographic appearance of the ovaries is imperative. Presence of a large, single, circular, fluctuating, and anechoic fluid-filled structure ~ 25 mm in diameter with a very thin outer wall (Figure 1A) in a normal size ovary allows a presumptive diagnosis of ovarian FC. Repeated examinations, in the absence of a response to the common treatment of ovarian cysts, or the presence of an oversize ovary should suggest a more meticulous and exhaustive differential diagnosis examination. Determining the size of the abnormal ovary is very important for the differential diagnosis. For OH, OA, and GCT, the ovaries were > 8 cm long. In contrast to cattle, most of the ovaries with GCT in human remain within normal size.[34] In the present study, GCT volume was more prominent than that of the OH or OA. With the increase of the ovarian mass, tissue metabolism and vascularization become more important. Evaluation of the blood supply around the ovary is crucial in deciding on the surgical approach to avoid the risk of fatal hemorrhage. The presence of adhesions, in the case of OA, may help in the differential diagnosis but again will force the surgeon to a cautious, meticulous, and adopt a stepwise surgical approach. Abscess may to be attached to surrounding structures, such as the rectum, the intestinal tract, or the uterine horn, reducing the ovary's mobility. Whereas this is an important observation for prognosis and surgical management, adhesions to the rectum are sometime difficult to assess by transrectal palpation.

As a result of variation in the ultrasonographic appearance of LOS anomalies, ultrasonography alone cannot guarantee an accurate diagnosis, although it is still an important technique for

establishing the right diagnosis. In humans with a larger variety and number of ovarian tumors, ultrasonography has high sensitivity (84 - 89%) and specificity (98.2%), but low positive predictive value (35.3%).[35] Ultrasonography allows the clinician to rule out additional anomalies in other organs or tissues, e.g. iliac and iliofemoral lymph nodes, the cervix, and the uterus with a higher degree of certainty. Evidence of some follicular activity in a fairly normal sized contralateral ovary (static) on ultrasonography help differentiate OA and OH from GCT in which contralateral ovary is atrophied. In the present cases, short-time hospitalization made the assessment of the follicular function difficult. Unfortunately, there is no clear and definitive ultrasonographic pattern of GCT. In mares, for example, its appearance varies from enlarged multicystic structures to a solid mass or single large fluid-filled cyst.[36] Sometimes, the affected ovary may have the typical honeycomb appearance on ultrasonography. Thus, the ultrasonographic appearance of GCT can be confusing with other types of ovarian tumors and LOS conditions.

In contrast to manual transrectal palpation and ultrasonography of the ovary, hematological and biochemical analysis were unreliable for diagnosing LOS, even in the case of OA. Our hematological results showed a simple pattern of stress, and the changes on the biochemistry panel were not significant. However, the hemato-biochemistry profiles of affected animals are still important for revealing underlying diseases that are not discovered during physical examination but could have an impact on the surgical management and prognosis as well as long-term reproductive efficiency. In the present study, all cases were negative for leukosis except for 1 animal with OA. However, studies in cattle had no clinical association between bovine leukosis virus infection and the incidence of the ovarian tumors or other conditions.[37]

Hormonal tests could be useful diagnostic tools in the differential diagnosis of LOS and, more specifically, in cases of hormone-secreting tumours like GCT. Biomarker like AMH for bovine GCT diagnosis has the highest diagnostic accuracy and sensitivity. An AMH concentration of ≥ 0.36 ng/ml had a sensitivity of 100% and specificity of 99.1%. The literature mentions that elevated serum inhibin (> 0.69 ng/ml) is a marker for ovarian GCT. This finding supports the hypothesis that the secretion of follicle-stimulating hormone (FSH) from the anterior pituitary is suppressed, probably due to the overproduction of inhibin and androgens by neoplastic cells.[38] Similar studies in the mare had elevated serum inhibin and testosterone concentrations in 87 and 54%, respectively. Like in the mare, sensitivity of AMH for detecting GCT was significantly higher than inhibin or testosterone. An AMH profile could also be part of patient monitoring for disease remittance after removal of GCT. After hemiovariectomy, AMH declined rapidly to normal concentrations within 1 week postsurgery.[39] In the present study, no AMH measurement were performed to confirm the final diagnosis of GCT before and after surgical removal of the LOS because of cost and time lag for the results. In the case where a GCT is suspected in a smaller size ovary, assessing the AMH would be an essential diagnostic tool. In cases of OA and OH, it is expected to have in normal physiological concentrations of AMH.

Finally, histopathology remains the main definitive diagnostic test for LOS and, more specifically, for ovarian tumors with the highest specificity (99.8%). OH is more common in mares, and it can be differentiated from GCT based on physical examination and profiles of hormones like AMH.[40]

All LOS diagnostics of the present report were confirmed by histopathology. The mitotic index for all but 1 case of GCT indicated no malignancy; however, malignancy does not necessarily indicate the invasive nature of the GCT in the cow. Histology of OH revealed that a large proportion of ovarian parenchyma samples were occupied by several large haematoma and necrotic coalescence, accompanied by extensive exudation of fibrin, red blood cells and degenerate neutrophils.

Conclusion

In general, LOS remains a rare anomaly. Large size ovary is more likely to be associated with GCT than with OA or OH. Meticulous transrectal palpation and ultrasonographic examination are essential to diagnose LOS in order to assess the form, size, localization, consistency, mobility, and the appearance of the ovaries. Although the ultrasonographic appearance of LOS varies, ultrasonography still represents a very important diagnostic tool for ruling out and characterizing other anomalies of the reproductive tract. The AMH assessment is a good tool to confirm the diagnosis of GCT but the cost and time lag for the results remains a major concern. However, with a suspicion of GCT with a small ovary on farms, assessment of AMH concentration is recommended. Hemiovariectomy of a large ovary (GCT, OH, or OA) has a very good short- and long-term prognosis, however, undiagnosed and untreated concomitant anomalies compromise the future fertility of the animal.

Conflict of interest

Authors do not have any conflict of interest to declare.

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