Surgical aspiration of a 19 centimeter ovarian follicular cyst in an embryo donor cow

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Summary

A two and a half year-old Holstein cow presented for assessment of a markedly enlarged ovary, with a fluid-filled structure measuring approximately 19 centimeters in diameter following superstimulation with follicle stimulating hormone (FSH) for embryo collection and transfer. Clinical evaluation, including transrectal palpation and ultrasonography, measurement of steroid hormone concentrations in serum and follicular fluid, and histopathologic examination of a sample obtained from the wall of the follicle-like structure during surgery were consistent with an ovarian follicular cyst. Surgical drainage of the fluid combined with medical treatment was effective in inducing cyclicity and did not result in complications (adhesion formation). New follicular cysts occurring within two weeks following the surgical aspiration were responsive to treatment with transvaginal ultrasound-guided aspiration followed by a 7-day CIDR-Synch protocol. The cow returned to cyclicity and subsequently became pregnant, producing twin bull calves within a year of initial presentation.

Background

While cystic ovarian degeneration (COD) is a relatively common disorder, particularly in cows subjected to superovulation protocols, this case is notable in the unusual size of the ovarian cyst. It demonstrates that surgical drainage may be an effective treatment in cows with markedly large ovarian cysts that preclude drainage via transvaginal ultrasound-guided aspiration or do not respond to hormonal therapies.

Case presentation

A two and a half year-old Holstein cow admitted to the Ohio State University Veterinary Medical Center Theriogenology Service for evaluation of an enlarged left ovary approximately four months after calving. The cow was previously enrolled in an embryo transfer program that included conventional treatments with FSH (Folltropin®-V; 16mL total equivalent to 320 mg of NIH-FSH-P1 in decreasing doses over four days) to induce superovulation for embryo collection and transfer. No reproductive examination (transrectal palpation or ultrasound) was performed before beginning superstimulation with FSH beginning 102 days post-calving. At the time of embryo collection attempt, both ovaries were classified as having corpora lutea too numerous to count based on transrectal palpation. In addition, the left ovary was greatly enlarged (cantaloupe-sized) and the right ovary was classified as cystic; nonetheless, the uterus was lavaged and sixteen embryos were recovered; eleven of transferrable quality (six grade 1, five grade 2) and five degenerate embryos. Nine embryos were transferred to appropriate recipients and two were frozen. Having failed to respond to conventional treatment utilizing gonadotropin releasing hormone (GnRH) and prostaglandin F2α (PGF; details of regimen employed not provided by owners or referring veterinarian), the cow was referred to the Ohio State University Veterinary Medical Center approximately one month after embryo collection.

The initial examination revealed an apparently healthy animal in good body condition, and blood chemistry and complete blood count were within reference ranges. On palpation per rectum, both ovaries were appreciably enlarged and soft. Palpation per rectum revealed the left ovary was markedly increased in size and could not be grasped in its entirety; the right ovary, albeit smaller than the left ovary, also contained cystic structures. On transrectal ultrasonography (Aloka SSD 500 V ultrasound scanner equipped with a 5.0 MHz linear array transrectal transducer; Aloka Co. Ltd., Wellingford, CT), the left ovary consisted mainly of a large fluid-filled structure, consisting of thin walls containing hypoechoic fluid (Figure 1). The left ovary could not be observed completely on the ultrasound monitor owing to the size of the cyst, but its diameter was estimated at approximately > 13 centimeters using the ultrasound

calipers (13 cm maximum depth allowed in the ultrasound unit used). The right ovary also contained two follicular cysts, measuring 3.1 and 3.0 cm in diameter.

Differential diagnosis

The most likely cause of ovarian enlargement on the left and right ovaries based on history and findings of the clinical reproductive examination was COD or ovarian follicular cysts. Other potential diagnoses considered included luteal cysts, ovarian neoplasia, and ovarian abscess.

Treatment

A presumptive diagnosis of bilateral follicular cysts was made and 5000 IU of human chorionic gonadotropin (hCG) was administered intravenously to induce luteinization of the cystic structures. Although GnRH or its analogues can also be administered for the same pharmacologic effect, hCG was elected due to its ability to bypass the adenohypophysis and bind directly to luteinizing hormone (LH) receptors in the ovary; the authors often elect this agent for chronic cases of COD that fail to respond to treatment with GnRH.

Transvaginal ultrasound-guided aspiration of the right ovarian follicular cyst was attempted. One cyst was successfully drained and the second cyst spontaneously ruptured during the manipulation per rectum. Owing to the large size of the left ovarian cyst, which precluded adequate manual manipulation per rectum, a decision was made to not attempt to aspirate it using transvaginal ultrasound-guided aspiration and a left flank laparotomy was elected to better access the left ovary and to ensure complete drainage of the accumulated fluid. Following left flank laparotomy, the left ovary was fully exteriorized to obtain diagnostic samples and facilitate drainage (Figure 2). Samples of the cystic fluid and cyst wall were collected for hormone analysis and histopathology, respectively. Biopsy samples were collected by insertion of an endoscopic biopsy instrument through a puncture created with a 14-gauge needle to drain the cystic fluid. After complete drainage of the fluid contained within the cyst, the ovary was repositioned within the abdominal cavity and no further resection of the cyst walls was performed; the abdominal wall and skin were closed in routine fashion. Post-operatively, multiple doses of ceftiofur (Excede™, Pfizer Animal Health, New York, NY; 6.6 mg/kg SC at the ear base weekly for 4 doses total) were prescribed to reduce the risk of oophoritis.[*]

The wall of the cyst was composed of loosely arranged fibrous tissue of varying degrees of maturity (Figure 3). Small arterioles and capillaries were interspersed within this tissue. The luminal aspect of the cyst was lined by multiple layers of round to cuboidal cells with slightly basophilic cytoplasm and round nuclei containing dispersed chromatin. There was no evidence of luteinized tissue, inflammation or neoplasia. Additional sections were submitted for vimentin and cytokeratin immunohistochemical staining. Rare cytokeratin staining was observed in the lining cells. Strong staining for vimentin was observed in the spindle shaped stromal cells as well as the cells lining the cyst described above (Figure 3). The morphology and immunohistochemical staining pattern suggested that the lining of the cyst was composed of granulosa cells. The expression of the intermediate filament proteins, vimentin and cytokeratin in ovarian structures can vary between species and stage of follicular development. In cattle, ovarian surface epithelium demonstrate positive cytokeratin and negative vimentin staining while rete ovarii demonstrate dual staining for cytokeratin and vimentin.[1] However, while positive vimentin staining has been shown to be a consistent finding in granulosa cells, keratin staining appears to be restricted to preantral follicles.[2] Thus, the staining pattern demonstrated supports the diagnosis of a cystic ovarian follicle.

Serum and follicular fluid samples collected at the time of surgery were submitted to the University of California-Davis Endocrinology Laboratory and were analyzed by enzymeimmunoassay (progesterone) and radioimmunoassay (estradiol, inhibin, testosterone). The results obtained for both serum and follicular fluid are listed in the Table.

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[*]At the time of case presentation, the described use of Excede was within AMDUCA guidelines. As of April 5, 2012, extra-label use of ceftiofur in disease prevention in food animals is prohibited by the Food and Drug Administration.

Thirty three days following surgery, the cow was again presented for a reproductive examination. On transrectal ultrasonography, the left ovary contained a 4.0 cm follicular cyst. The right ovary had a 2.5 cm cavitated corpus luteum and a 2.5 cm cystic follicle. No adhesions were detected. At that time, a single injection of a GnRH analog (1.8 mg of deslorelin acetate) was administered intramuscularly. One week later, the cysts were still present without ultrasonographic signs of ongoing luteinization and measured approximately 4.1 cm and 2.3 cm on the left and right ovaries, respectively; the corpus luteum was still present on the right ovary and concentrations of serum progesterone were 3.5 ng/mL. Transvaginal ultrasound-guided follicular aspiration was performed, and 25 mL of follicular fluid was aspirated from the left ovary and 6 mL from the right. The owner was instructed to place a controlled internal drug-releasing insert (CIDR) for seven days, administer PGF at removal, and breed when the cow was seen in estrus.

Outcome

The owners reported that following the final treatment, the cow returned to cyclicity and became pregnant approximately two months following surgery. The following year, she produced twin bull calves but succumbed to severe pneumonia within a week of parturition.

Discussion

The case described in this report is notable in the marked size of the follicular cyst and the successful restoration of reproductive function following surgical aspiration. Cystic ovarian disease occurs relatively frequently, affecting approximately 10% of dairy cows annually;[3] true incidence may actually be higher as a proportion of cows recover spontaneously, and some herds reportedly experience higher prevalence of this disorder. The economic impact of this condition is significant, as, on average, it prolongs inter-calving interval by 50 or more days[3] and may necessitate culling animals unresponsive to treatment. Due to its prevalence and impact on production, this disorder is very important to the dairy industry.

Traditionally, ovarian follicular cysts have been defined as anovulatory fluid-filled structures greater than 24 mm in diameter that persist for more than 7-10 days in the absence of a normal corpus luteum (CL);[4] however, cysts may also occur in conjunction with a CL.[5] Large cysts, up to 5 cm in diameter, have been described.[5] Ovarian cysts are relatively common in the postpartum period, with the highest incidence 30-60 days after parturition.[7] Application of ultrasonography for reproductive evaluation and enhanced understanding of bovine follicular dynamics have contributed to knowledge regarding cystic ovarian disease by allowing serial monitoring and more accurate documentation of ultrasonographic characteristics. The pathological condition actually entails a dynamic process with three potential circumstances: a single cyst may persist, the cyst may spontaneously regress (20-50%), or cysts may be cyclically replaced by others.[8-10] Follicular wave turnover takes longer (13.0 ± 1.1 days) in cystic cows as compared to normal, cycling animals (8.5 ± 0.5 days).[10] It is noteworthy that superstimulation with exogenous of FSH in the cow described in this case was implemented in the postpartum period without reproductive examination prior to initiation of treatment; therefore, it is possible that there was some pre-existing cystic condition that was exacerbated by hormonal treatment.

Despite the failure to characterize the etiological basis of COD, neuroendocrine dysfunction and alterations of the hypothalamic-hypophyseal-ovarian (HHPO) axis appear to play a significant role in the pathogenesis. A fundamental disruption in normal endocrine function and feedback mechanisms is apparently involved in the pathophysiology,[3,11,12] specifically, failure of positive feedback of estradiol-17β to the surge center of the hypothalamus. Cows with cystic ovaries show no or delayed LH response to the administration of exogenous estradiol compared to normal animals.[12,13] At the hypothalamic level, assessment of GnRH concentrations in homogenates of hypothalamic-suprachiasmatic nuclei from cystic cows revealed lower content in cystic versus control animals and there tended to be higher content in the median eminence of cystic cows.[14] Increased release of GnRH from the median eminence could account for higher serum concentration of LH and more frequent peaks in cystic as compared to normal cows; despite these changes, a preovulatory LH surge is not achieved.[10,14] While failure of positive feedback of

estradiol-17β and subsequent absence of an LH surge is widely accepted to be the underlying endocrine event leading to cyst formation, there is some evidence that animals chronically affected with COD may display similar LH pulsatility profiles compared to normal animals.[15] By contrast, FSH levels do not differ in cystic versus control cows, and cystic cows show a normal increase in FSH prior to initiation of a follicular wave.[10,14]

Administration of FSH to cows with chronic COD results in increased expression of LH and FSH receptors in ovarian tissue and increased intrafollicular estradiol-17β compared to untreated cows.[15] The administration of supra-physiological levels of FSH as commonly done in superovulation protocols may contribute to the development of COD. In one study, incidence of COD in beef embryo donor cows in Japan was 20% compared to a reported rate of 4.2% in normal dairy cows in the same area.[16]

Ovarian steroid expression in follicular cysts may be altered as compared to normal follicles. Expression of gonadotropin receptors and steroidogenic enzymes is altered in the development of cysts as compared to normal follicles.[17] Cows with cystic ovaries generally have elevated serum concentrations of estradiol-17β as compared to normal cows.[10,14] The cow described in present report did not show elevated steroid hormone levels in serum, and estrogen concentrations were low in follicular fluid as compared to normal developing follicles. It is important to note that different cysts may exhibit different steroid hormone profiles, and various classification systems have been derived according to these characteristics. Actual concentrations of serum and follicular steroid hormones in normal and cystic animals have been reported previously;[15,17,18] however, the reported values show marked variation among individuals and even within the same animal in addition to different approaches and hormonal assays.[19] Therefore, consideration of the ratio of hormone expression rather than the actual values[20] or total steroid hormone concentration (calculated based on follicle diameter)[17] provide alternate approaches in interpretation of results. For the cyst described here, the concentration of estradiol-17β in the follicular fluid was relatively low (86.4 pg/ml), but the total content (310 ng) was significant when accounting for total follicular fluid volume [based on the volume of a sphere according to the formula $\frac{4}{3}*(\pi r^3)$]. This value is within the range reported for total estrogen in follicular cysts.[21] While it is interesting to consider these physiological parameters and characterize the cyst according to its hormonal profile, it might be prudent to consider that COD is a dynamic process and that such information on steroid composition of the follicular fluid may only provide a snapshot of the cyst endocrine function at the time of sampling.

Due to the apparently defective GnRH secretion and LH surge in cows affected by cystic ovarian disease, medical therapy has aimed at use of these hormones or their analogs to induce luteinization. Administration of hCG was originally employed with reported success varying from 65-80%,[9] and this was administered on initial assessment in the case presented here because of the previous history of unsuccessful GnRH use. The use of hCG has increasingly been replaced by GnRH and GnRH analogs with reported success rates of about 80%.[9] Because these approaches aim to luteinize the cystic structure, administration of PGF seven to nine days following GnRH or GnRH analog administration may shorten the interval between initiation of treatment and estrus. An alternate approach employs the use of intervaginal progesterone-releasing devices and ovulation or estrus synchronization protocols as treatment to increase the chances to return to cyclicity. Pretreatment with GnRH increases the number of cows with a CL at the time of prostaglandin administration, and insertion of a progesterone releasing device results in acceptable pregnancy rates in treated cows.[22] Furthermore, management of cows with a synchronization protocol employing a progesterone releasing device results in decreased incidence of COD compared to previous occurrence using PGF only.[16]

The use of transvaginal needle aspiration of cystic fluid has been more recently employed as a treatment for follicular cysts,[23] and it can be effective when employed even in cows who are unresponsive to traditional treatment with synchronization protocols.[24,25] Transvaginal ultrasound guided aspiration of follicular cysts may be considered as a modification and improvement on the older approach of manual rupture of ovarian cysts in that it carries a decreased risk of adhesions and the added benefit of acute removal of hormone-rich cystic fluid and, therefore, potentially fast recovery and resumption of normal cyclicity. Cairoli et al. reported a 75.6% recovery rate and 64.7% conception rate when aspiration was combined with administration of the GnRH analogue buserelin.[23] In another study, 100% of cystic cows

treated with transvaginal ultrasound guided aspiration in combination with GnRH and PGF recovered, including animals which did not respond to aspiration alone (5.5%) or GnRH and PGF alone (16%) initially; the average interval from treatment to estrus was 12.4 ± 1.1 days.[24] Thus, transvaginal aspiration of follicular cysts may be considered in animals that seem refractory to GnRH and PGF. In the case reported here, surgical aspiration was elected initially to allow drainage of the very large structure, and to obtain a biopsy sample for histopathologic examination. Thirty-three days following the initial surgical aspiration, bilateral ovarian cysts and a cavitated CL were diagnosed and the cow was treated with an intramuscular injection of 1.8 mL deslorelin acetate. On re-evaluation a week later, the cysts were still present and drained via transvaginal ultrasound-guided aspiration; no other follicular structures were detected at the time of the transvaginal aspiration. Because the removal of follicles via ultrasonographic ablation should elicit the emergence of a new follicular wave, the cow was then subjected to a 7-day CIDR-Synch protocol.

Although ovarian follicular cysts are not an infrequent occurrence in dairy cattle, this case is remarkable due to the sheer size of the lesion. In the present case, it is probable that the serial administration of exogenous FSH to induce superovulation contributed to the marked growth of the cystic follicle beyond the typical size reported for follicular cysts. This experience may therefore emphasize the need to closely examine cows undergoing superovulation protocols, especially in the post-partum period, and to provide prompt intervention when cows develop COD and remain unresponsive to conventional hormonal treatments.

Learning points
- Cystic ovarian disease is a frequent occurrence with dramatic economic impact on the dairy industry. The administration of exogenous FSH in superovulation protocols for embryo transfer can contribute to increasing incidence and severity of this process. Embryo donor cows subjected to FSH superovulation protocols should be monitored for developments of cysts during FSH treatment or shortly after. In our clinic, a subsequent examination of the ovaries is performed with ultrasonography at the time of embryo collection.
- While most animals with COD respond to medical treatment (administration of a GnRH analogue or hCG, in conjunction or not with an intervaginal progesterone releasing device and PGF), transvaginal aspiration and removal of cystic contents has been shown to be successful in refractory cows that do not respond to conventional hormonal treatments; transvaginal aspiration in association with hormonal treatments is currently the method of choice for treatment of COD in our clinic. The present case illustrates that surgical drainage and removal of cystic contents may be warranted in cows afflicted with unusually large ovarian cysts that preclude adequate ovarian manipulation per rectum and transvaginal ultrasound-guided aspiration, and that have not responded to conventional hormonal treatments.

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Figure 1. Ultrasonograph of the left ovary. The ovary was markedly enlarged (too large to be effectively imaged with a 5.0MHz linear transducer probe) with a single cystic structure, filled with hypoechoic fluid. (Aloka, 5.0MHz linear transducer, hash-marks represent 1cm)

Figure 2. Left flank laparotomy. Exteriorization of the ovary allowed observation of a large cystic structure (19 cm in diameter) with distended, well-vascularized wall. Fluid contents were straw-colored.
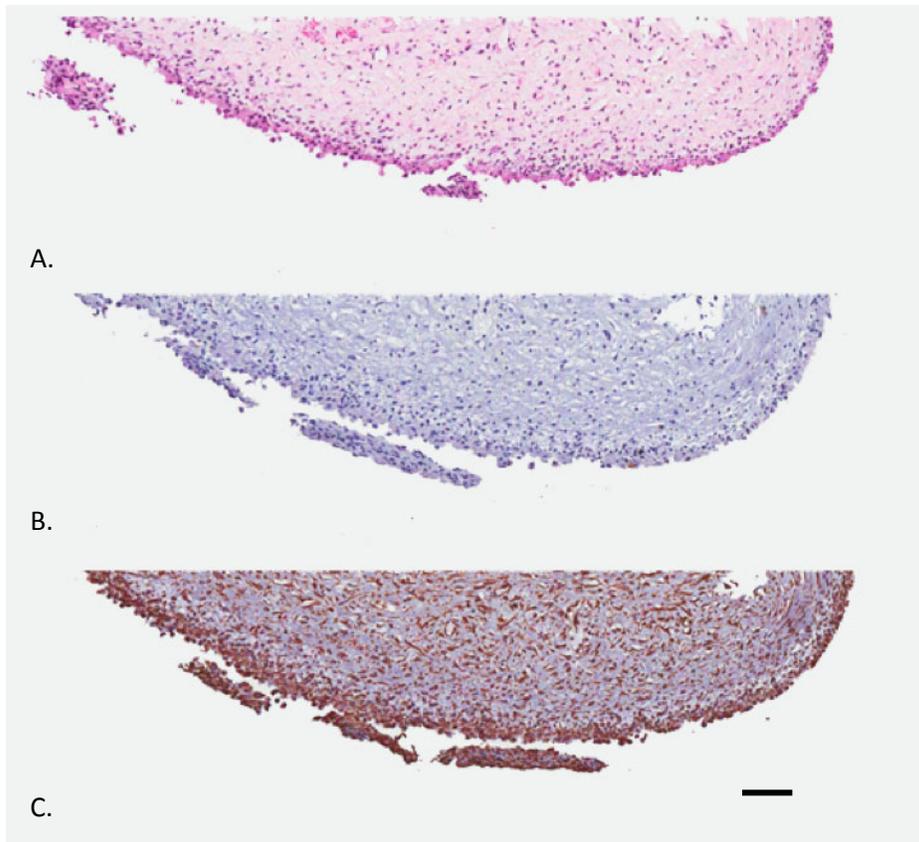

A.

B.

C.

Figure 3. Immunohistochemical staining profile of tissue submitted from the ovarian cyst. A. Hematoxylin and eosin stain. B. Cytokeratin stain. C. Vimentin stain. The brown color indicates positive cytoplasmic staining. Bar represents 100 μm.

Table: Concentration of hormones in serum and follicular fluid collected at the time of flank laparotomy.

Hormone	Serum	Follicular Fluid
Progesterone (ng/ml)	0.2	76.0
Estradiol (pg/ml)	24.2	86.4
Testosterone (pg/ml)	22.7	45.4
Inhiin (ng/ml)	1.1	>10.0

(Editor's Note: The photographs in this paper appear in color in the online edition of Clinical Theriogenology.)