PEER REVIEWED

Monitoring Return to Cyclicity Following Removal of a Granulosa Cell Tumor Associated with Precocious Lactation in an 11-month-old Holstein Heifer

Colin W. Palmer*, *DVM, MVetSc, Dip ACT*[1]; **Edward G. Clark,** *DVM, MVetSc*[2]; **Terry D. Carruthers,** *DVM, PhD*[1]

[1]*Department of Large Animal Clinical Sciences, University of Saskatchewan, Saskatoon, Sask., Canada S7N 5B4*

[2]*Prairie Diagnostic Services, University of Saskatchewan, Saskatoon, Sask., Canada S7N 5B4*

**Corresponding author: tel: 1-306-966-7150; fax: 1-306-966-7159; E-mail address: colin.palmer@usask.ca*

Abstract

An 11-month-old Holstein heifer was presented to the Western College of Veterinary Medicine for evaluation of precocious lactation associated with a palpably enlarged right ovary. Ultrasound examination of the ovaries revealed a 6 inch (15 cm) diameter right ovary with a multilobulated appearance, and a small left ovary measuring 0.6 inches (1.6 cm) long by 0.3 inches (0.8 cm) wide. The mammary secretion was consistent with milk: fat – 3.07%, protein – 4.78%, and lactose – 1.07% with a somatic cell count of 4.8 x10^6/ mL. Prior to removal of the right ovary by right flank laparotomy, serum hormone levels were measured: testosterone (0.01 ng/mL), estradiol-17β (9.1 pg/mL), progesterone (0.2 ng/mL), prolactin (5.8 ng/mL), luteinizing hormone (0.14 ng/mL) and follicle stimulating hormone (0.32 ng/mL). Gross examination revealed that the right ovary weighed 7.34 oz (208 g) and was composed of numerous cysts (0.2 – 1.6 inches [0.5 – 4 cm] in diameter) containing fluid or blood. Microscopically, foci of cells of a follicular or luteal nature were noted. The definitive diagnosis was a right ovarian granulosa cell tumor, and milk secretion declined rapidly following tumor removal. The left ovary was monitored ultrasonographically at weekly intervals to determine onset of cyclicity. Follicular activity resumed between three and four weeks post-surgery, followed by detection of a corpus luteum at day 32. The heifer became pregnant five months post-surgery, and has since delivered a live calf. This case represents only the second reported case of precocious lactation associated with a granulosa cell tumor, and suggests that cyclicity is likely to return to the contralateral ovary less than a month after tumor removal.

Résumé

Une taure Holstein de 11 mois a été reçue au *Western College of Veterinary Medicine* pour l'évaluation d'une lactation précoce associée avec un ovaire droit palpablement élargi. L'examen aux ultrasons des ovaires a révélé que l'ovaire droit avait un diamètre de 6 pouces (15 cm) et une apparence multilobulaire tandis que l'ovaire gauche était petit et mesurait 0.6 pouces (1.6 cm) de long et 0.3 pouces (0.8 cm) de large. La sécrétion mammaire ressemblait à du lait : gras – 3.07%, protéines – 4.78% et lactose – 1.07%, avec un comptage de cellules somatiques de 4.8 x 10^6 /ml. Avant l'excision de l'ovaire droit par laparotomie du flanc droit, le niveau sérique des hormones a été mesuré : testostérone (0.01 ng/ml), estradiol-17b (9.1 pg/ml), progestérone (0.2 ng/ml), prolactine (5.8 ng/ml), hormone lutéinique (0.14 ng/ml) et la folliculine (0.32 ng/ml). L'examen macroscopique indiquait que l'ovaire droit pesait 7.34 oz (208 g) et comportait plusieurs kystes (0.2 – 1.6 pouces [0.5 – 4 cm] de diamètre) contenant du fluide ou du sang. Au niveau microscopique, plusieurs foyers de cellules de type folliculaire ou lutéal ont été notés. Le diagnostic final était une tumeur cellulaire granulaire de l'ovaire droit. La sécrétion de lait cessa rapidement suite à l'excision de la tumeur. L'ovaire gauche a été examinée avec des ultrasons toutes les semaines pour déterminer le début de la cyclicité. L'activité folliculaire reprit place entre la troisième et quatrième semaine suivant la chirurgie et un corps jaune à été détecté au jour 32. La taure est devenue gestante 5 mois suivant la chirurgie et a mis bas à un veau vivant. Ce cas est seulement le deuxième cas rapporté d'une lactation précoce associée avec une tumeur cellulaire granulaire et suggère que la

cyclicité peut prendre place dans l'ovaire opposé moins d'un mois suivant l'excision de la tumeur.

Introduction

There is only one other reported case of precocious lactation associated with a granulosa cell tumor in a heifer.[8] Chronic treatment with estrogens, estrogens and progesterone, or estrogen after progesterone exposure all induce lactation to some extent.[3] Granulosa cell tumors are frequently hormonally active, producing estrogens, progesterone or even androgens, and may vary in type and quantity of hormone produced over time.[4] In nearly all cases, atrophy of the contralateral ovary occurs, most likely because of inhibin production by the tumor and consequent suppression of follicle stimulating hormone. According to one source, the atrophic ovary will likely regain normal function within a year of removal of the tumor; however, no specific case references were cited.[4] The prognosis for return to cyclicity is a frequently asked question, but there is a paucity of case-based information in the literature. The objectives of this clinical evaluation were to investigate the cause of the precocious lactation, definitively diagnose the reason for the enlarged ovary, investigate the hormones being produced by the enlarged ovary, and to monitor the heifer for return to cyclicity following ovariectomy.

Materials and Methods

An 11-month-old, 805 lb (365 kg) Holstein heifer was referred to the Western College of Veterinary Medicine for evaluation of precocious lactation accompanied by a palpably enlarged right ovary. A physical examination, complete blood count, serum chemistry profile and urinalysis comprised the initial investigation. Serum testosterone, estradiol-17β, progesterone, prolactin, luteinizing hormone and follicle stimulating hormone concentrations were determined using validated radioimmunoassay. A composite sample of mammary secretion was evaluated for fat, protein, lactose and somatic cell content. Both the left and right ovaries were scanned ultrasonographically using a 5.0 MHz linear-array transducer,[a] enabling the recording of detailed measurements and an evaluation of ovarian composition.

The right ovary was removed via a right-flank laparotomy and submitted for pathological analysis. The ovarian pedicle was double ligated and observed for hemorrhage prior to routine abdominal closure. Postoperative treatment consisted of 500 mg of ceftiofur sodium[b] IM for five days.

Following surgery, ultrasonographic monitoring of the left ovary continued using a 7.5 MHz linear-array transducer[c] at approximately weekly intervals, beginning seven days after surgery and continuing until there was a noticeable change in follicular activity. Specific parameters of interest included length and width of the ovary and diameter of the largest follicle. In an attempt to monitor the ovary for ovulation and the formation of a corpus luteum an additional ultrasonic examination was performed three days (day 32 post-surgery) following the first change in follicular activity. The heifer was discharged to the farm of origin and monitored for expression of estrus. Artificial insemination was performed at two different estrus periods and pregnancy status was confirmed by rectal palpation at 60 days post-breeding.

Results

Physical examination revealed a heifer in good condition with a normal vulva and vagina. Body temperature, heart and respiratory rates were all within normal range. The udder was enlarged and what appeared to be milk was leaking from the teats, especially the left hind. The initial ultrasound examination revealed an enlarged right ovary approximately six inches (15 cm) in diameter with a multilobulated appearance; the largest cystic cavity measured 2.2 inches (5.6 cm) in diameter. The left ovary measured 0.6 inches (1.6 cm) long by 0.3 inches (0.8 cm) wide.

Urinalysis was normal and the serum chemistry profile was unremarkable except for a slight elevation in calcium (10.7 mg/dL, normal – 9.14-10.5 mg/dL) and sorbitol dehydrogenase (23.0 U/L, normal – 4-18 U/L). The complete blood count was indicative of a stress leukogram with an increase in segmented neutrophils, 6.958 x 10^9/L, and a slight decrease in lymphocyte numbers to 2.450 x 10^9/L. Eosinophil numbers remained in the low-normal range (0.098x10^9/L). Table 1 shows the results of serum hormone analysis.

The endocrine profile was unremarkable; however, it is noteworthy that the progesterone level was low at 0.2 ng/mL and estradiol 17β was 9.1 pg/mL. The udder secretion was consistent with milk: fat – 3.07%, protein – 4.78%, lactose – 1.07% and the somatic cell count was 4.8 x 10^6/mL.

Gross examination of the right ovary indicated that it weighed 7.34 oz (208 g) and was composed of a large central cavity (Figure 1) and numerous peripheral cystic cavities ranging in size from 0.2 to 1.6 inches (0.5 to

Table 1. Results of serum hormone analysis.

Progesterone (ng/mL)	0.2
Testosterone (ng/mL)	0.01
Estradiol 17β (pg/mL)	9.1
Prolactin (ng/mL)	5.8
Luteinizing hormone (ng/mL)	0.14
Follicle stimulating hormone (ng/ml)	0.32

Figure 1. Cut surface of the right ovary (granulosa cell tumor).

4 cm). Most of these cysts contained clear fluid or blood. Microscopically, the tissue sections consisted of round foci within a connective tissue stroma. The foci contained combinations of cells, blood and empty space. Based upon the types of cells present, many of the foci were interpreted to be mature and secondary follicles whereas other foci were interpreted to be corpora lutea. Blood-filled foci were either lined with an endothelium or were dilated vascular spaces.

Milk production declined rapidly post-surgery and the udder became progressively smaller. Results of the serial ultrasound examinations of the left ovary are shown in Table 2. Follicular activity resumed between three and four weeks post-surgery. The heifer became pregnant to an insemination four months later and has since delivered a live calf.

Discussion

Granulosa cell tumors are the most common ovarian neoplasia of cattle[1,5,9] and have been reported in all age groups.[1,2,4,5,8] Precocious lactation associated with a granulosa cell tumor, however, has been reported only once.[8] Those authors noted that serum estrogen (1446 pg/mL) and progesterone (5.6 ng/mL) concentrations were elevated and declined shortly after tumor removal.[8] Udder development and subsequent lactation apparently require elevated levels of estrogen and progesterone for a prolonged duration.[8] Treatment with progesterone and estradiol 17β for periods of as little as seven days has been effective in inducing lactation,[3] but it is well known that the fluctuations in progesterone and estrogen associated with cyclicity are not enough to induce mammary development.[3,7] Estradiol 17β and progesterone levels recorded in the present case were unremarkable; much lower than those reported in the previous case. Elevated progesterone inhibits the positive effects of prolactin and glucocorticoids on milk production and must decline to enable the onset of lactation.[6] It seems possible that if serum samples were collected in the weeks prior to the initial presentation of this heifer that much higher levels of at least estradiol 17β would have been found. Prolactin levels were not measured in the previous case and were considered to be within normal range in this case. Prolactin does play a role in mammary development[6] and the onset of milk production, but does not appear to be essential for the maintenance of lactation.[3,6] Levels of prolactin such as those reported in this case likely have little effect on the maintenance of lactation. We did not consider measuring glucocorticoid levels in our workup; however, we can conclude that the granulosa cell tumor and milk production were linked as milk production declined rapidly following tumor removal.

Atrophy of the contralateral ovary is commonly associated with granulosa cell tumors. Conclusive evidence implicating testosterone as the cause is lacking. Hormone production associated with these tumors is variable and in many cases, including the present case, testosterone levels are low. The current dogma is that inhibin production by the tumor cells is responsible for ovarian atrophy through its suppressive effects on follicle stimulating hormone.[4] Unfortunately, inhibin levels were not measured in this case, but would have been a valuable adjunct. One reference indicated that the atrophic ovary would return to function within a year

Table 2. Results of ultrasound examinations.

Days post-surgery	Ovary length, inches (cm)	Ovary width, inches (cm)	Follicular diameter, inches (cm)	CL diameter, inches (cm)
Day 7	0.78 (2.0)	0.35 (0.9)	0.04 (0.1)	-
Day 14	0.83 (2.1)	0.39 (1.0)	0.16 (0.4)	-
Day 21	0.91 (2.3)	0.39 (1.0)	0.20 (0.5)	-
Day 29	1.57 (4.0)	0.63 (1.6)	0.35 (0.9)	-
Day 32	1.46 (3.7)	0.94 (2.4)	0.51 (1.3)	0.83 (2.1)

following removal of the granulosa cell tumor. However, no specific cases were cited.[4] In this heifer, follicular activity resumed within 14 days of tumor removal with ovulation occurring before 30 days post-surgery. The investigators in the previous case also monitored follicular development on the contralateral ovary. That heifer appeared to develop a small follicle within 30 days of tumor removal, but it remained static and was treated as a cyst. Nevertheless, these two cases suggest that follicular activity can be expected to return much sooner than previously thought. The presence of both a corpus luteum and a large follicle on the left ovary of the current heifer on day 32 post surgery seems puzzling. Prior to day 32, it was thought that the development of a single follicle was being monitored. A possible explanation might be that ovulation had occurred between days 21 and 29, but the developing corpus luteum was still too immature to be detected by ultrasonography on day 29. The 0.35 inch (0.9 cm) diameter follicle noted on day 29 was perhaps the dominant follicle of a new follicular wave that had emerged two to three days prior.

Conclusion

This case represents the second reported case of precocious lactation associated with a granulosa cell tumor in cattle. The contralateral ovary is likely to return to normal function less than one month after granulosa cell tumor removal.

Footnotes

[a] Aloka SSD 500; Aloka Co., Wallingford, CT
[b] Excenel® Sterile Powder, Pharmacia Animal Health, Orangeville, ON, Canada
[c] Aloka SSD 900; Aloka Co., Wallingford, CT

References

1. Cotchin E: Spontaneous Tumors of the Uterus and Ovaries in Animals, in Cotchin E, Marchant J (eds): *Animal Tumors of the Female Reproductive Tract: Spontaneous and Experimental.* New York, Springer-Verlag, 1977, pp 26-65.
2. Dubra CR, de Briones EA, Long SE: A case of granulosa-cell tumour in a heifer. *Vet Rec* 91: 9-11, 1972.
3. Erb RE: Hormonal control of mammogenesis and onset of lactation in cows – a review. *J Dairy Science* 60: 155-169, 1977.
4. Kennedy PC, Miller RB: The female genital system, in Jubb KVF, Kennedy PC, Palmer N (eds): *Pathology of Domestic Animals*, ed 4, Vol 3. New York, Academic Press Inc, 1993, pp 349-470.
5. Sartin EA, Herrara GA, Whitley EM, Riddell MG, Wolfe DF: Malignant ovarian tumors in two heifers. *J Vet Diagn Invest* 8: 265-267, 1996.
6. Tucker HA: Symposium: hormonal regulation of milk synthesis: hormones, mammary growth, and lactation: a 41-year perspective. *J Dairy Sci* 83: 874-884, 2000.
7. Vestweber JG, Cole DE, Quadri SK, Schmidt MK: Premature mammary development in a heifer with abdominal mesothelioma. *J Am Vet Med Assoc* 189:804-805, 1986.
8. Whitacre MD, van Camp SD, Maclachlan NJ, Umstead JA: Premature lactation in a heifer with a sex cord-stromal tumor. *J Am Vet Med Assoc* 193: 946-948, 1988.
9. Zachary JF, Haliburton JC: Malignant granulosa cell tumor in an Angus cow. *Vet Pathol* 20: 506-509, 1983.

Abstract

The Role of Micronutrients in Bovine Periparturient Problems

Mee J.F.
Cattle Practice (2004) 12(2):95-108

Under commercial farming conditions in the British Isles, the micronutrients most likely to cause bovine periparturient problems are copper, iodine, molybdenum, selenium and vitamin E. The periparturient problems most commonly associated with these micronutrients are perinatal morbidity or mortality, retained fetal membranes (RFM), immunosuppression, nutritional muscular dystrophy (NMD) and illthrift. With the exception of NMD, micronutrient imbalances are not the most important causes of these problems. Strong experimental data support the relationships between RFM, mastitis, NMD and selenium/vitamin E and congenital defects with iodine, copper, manganese and vitamin A. Equivocal experimental data but strong case series data support the relationships between weak calf syndrome (WCS) and iodine/selenium and neonatal morbidity/illthrift with selenium/vitamin E and copper. Case series data support the relationships between abortion and iodine or selenium and abnormal calvings with selenium or iodine. The high incidence of copper, selenium and cobalt deficiencies has declined over the last two decades but the low incidence of iodine and vitamin A deficiencies has remained stable in the UK cattle population.