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Technical Report: Serial collections of placentomes during parturition in cattle and subsequent reproductive performance

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Technical Report: Serial collections of placentomes during parturition in cattle and subsequent reproductive performance[1]

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ABSTRACT: Placental separation is a complex physiological event in reproductive physiology and the underlying molecular mechanisms remain unclear. When comparing different experiments the timing of tissue collections is a significant consideration due to the variability in time between fetal expulsion and expulsion of the placenta (30 min to >24 h). This makes comparison of tissues samples across animals difficult and supports the need for serial tissue collections within animal. Additionally, the instrument most commonly used, a modified Richter-Resinsinger effeminator, for placentome collections is difficult to obtain and there are no data in the literature record regarding subsequent reproductive performance of animals subjected placentome collections. To facilitate continued research into the physiology behind placental separation, we designed an instrument from readily available components and performed serial transvaginal placentome collections in cattle. Three placentomes at 2-h intervals were collected after expulsion of the calf in 18 multiparous cows. There was no incidence of mortality and all cows resumed estrous after the procedure. Neither time from placentome collection nor age had a significant effect on pregnancy status at diagnosis ($P > 0.05$). These results demonstrate the viability of and utility of this device for collecting multiple placentomes in cattle.

Key words: placental separation, retained placenta, surgery, transvaginal

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INTRODUCTION

The molecular mechanisms responsible for placental separation are 1 of the least understood reproductive events in domestic animal physiology and occur during the last stage of parturition. In cattle, failure to expel the placenta for at least 12 h after expulsion of the calf is defined as retained placenta and is known to negatively impair the reproductive performance of afflicted animals (Eiler and Fecteau, 2007). Placental separation constitutes the last stage of parturition and there is considerable variation in the amount of time (30 min to >24 h) required for completion of stage 3 of parturition. Due to the complexity of endocrine changes during parturition and the variation in time required for placental expulsion, the method of tissue collection and the timing of collection are important considerations when comparing data from different investigators. Currently, the instrument most commonly used for placentome collections, a modified Richter-Resinsinger effeminator, is difficult to obtain (Sharpe et al., 1990; Maj and Kankofer, 1997; Streyl et al., 2012). Additionally, there are no data available in the literature documenting the effects of placentome collections on the morbidity, mortality, or subsequent reproductive performance of cattle. As the time required from calf expulsion to placental expulsion varies by animal, comparing tissue samples across animals is less than ideal. Serial tissue collections would eliminate this obstacle, yet ethical and economic concerns must be addressed before conducting large scale physiologic studies. This is especially true today where the costs associated with maintaining research herds of large domestic animals has resulted in the reduction of animal numbers maintained by universities. With these concerns in mind, the purpose

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of this technical report is to 1) document a methodology for serial collection of placentomes transvaginally, 2) provide plans for an inexpensive device for the collection of placentomes, and 3) report subsequent pregnancy rates investigators can expect from cows subjected to multiple insults (e.g., abortion of preterm calf, subjected to tissue collections).

MATERIALS AND METHODS

All procedures were conducted in accordance with the U.S. Meat Animal Research Center (**USMARC**) Institutional Care and Use Committees policies and guidelines regarding the care and use of vertebrate animals.

Animals

Across a 3-wk window, pregnant cull cows (n = 18; mean age = 10.8 yr) from the USMARC beef herd were induced to abort between estimated gestational day of 201 to 210 using 25 mg dexamethasone (Bimeda-MTC Animal Health Inc., Cambridge, Ontario, Canada) and 37.5 mg of $PGF_{2\alpha}$ (Lutalyse, Pfizer, Kalamazoo, MI) intramuscularly. Five cows aborted without assistance within 60 to 67 h after treatment. The remaining 13 cows were restrained in a squeeze chute and assessed for cervical dilation and presentation of the fetus at 60 ± 7 h posttreatment. If the cervix was dilated (n = 12), the calf was removed using veterinary obstetrical chains and euthanized. One cow did not dilate until 91 h, at which time her calf was removed and subsequently euthanized.

Instrumentation and Tissue Collections

Collection of placentomes was performed transvaginally using the placentome attainment device (**PAD**; Fig. 1a). The PAD consists of a 0.3-mm steel guitar string and a 10.0-cm length of brass tubing with a 3.0-mm o.d. and 0.5-mm wall thickness. Three holes, 1.0 mm in diam., were drilled completely through both sides of the tubing approximately 4.0 mm apart on center between 3.1 and 5.1 cm of the tubing (Fig. 1b). The tag end of the guitar string was passed through the brass grommet of the string and then through all 3 sets of holes. The string was held in place by the first loop of string and the handle. The device was capable of making at least 18 collections before failure of the string.

Three placentomes were collected 2 h apart. Before collection of a placentome, the tail head was clipped, scrubbed with iodine, and rinsed with 100% ethanol after which an epidural, consisting of 5.0 mL of 2% lidocaine (Aspen Veterinary Resources Ltd., Liberty, MO), was administered. The anus and vulva of the cow was then scrubbed with an iodine solution and the gloved hand and arm of the operator was passed into reproductive tract of the cow via the vagina. The collection loop of the PAD was carried into the reproductive tract perched on the thumb, index, and middle finger of the off hand of the operator. The desired placentome was then identified and the collection loop of the PAD was placed over the placentome and positioned at the base of the caruncle. The placentome was then held in place and increasing force was applied to the handle of the PAD, increasing the tension of the string and drawing the collection loop closed. This resulted in the laceration of the adjacent endometrium and most of the fetal membranes, leaving an isolated placentome. After the last collection, flunixin meglumine (SUPPRESSOR; 1.1 mg/kg BW; Merck, Summit, NJ) was administered intravenously along with ceftiofur (EXCEEDE, 6.6mg/kg BW; Pfizer, New York, NY), subcutaneously, at the base of the ear. No further interventions were administered.

Sixty days after the last set of placentome collections, the reproductive tracts of cows were examined via ultrasonography to check for lesions of the reproductive tract and evidence of resumption of estrous using the reproductive tract score (**RTS**) system described by Mee et al. (2009). After this examination, cows were penned with bulls (n = 2) of proven fertility for 63 d and pregnancy status was determined via ultrasonography 42 d after removal of the bulls. Gestational age was estimated using ultrasonography by 2 experienced operators.

Statistical analysis was performed using the MIXED procedure (SAS, Inst. Inc., Cary, NC) with pregnancy status as the dependent variable and involution interval (days from tissue collection to bull exposure) and age of dam as independent variables. Differences were considered significant when $P \leq 0.05$.

RESULTS AND DISCUSSION

At the 60 d ultrasound, 100% of the cows met the criteria for an RTS 1 and no lesions (adhesions and/or metritis) were detected. At pregnancy diagnosis, 11 of the 18 (61%; median gestational age = 90 d; range 50 to 100 d) cows were pregnant and there was no effect of involution interval (defined as the number of days from last tissue collection to day of first bull exposure; P = 0.76) or dam age (P = 0.52) on pregnancy status. Of the 7 cows who were open at pregnancy diagnosis, 5 cows appeared to be normal (e.g., no abnormalities detected), 1 cow had an enlarged uterine horn, which was not present during previous examination (possible aborted early pregnancy), and 1 cow was lame during the breeding window, making her unlikely to stand to be bred by the bull.

A comparison of pregnancy data with data from previous reports is difficult. To our knowledge, pregnancy rate after elective abortion is not typically generated or reported. Two of the most applicable reports are from

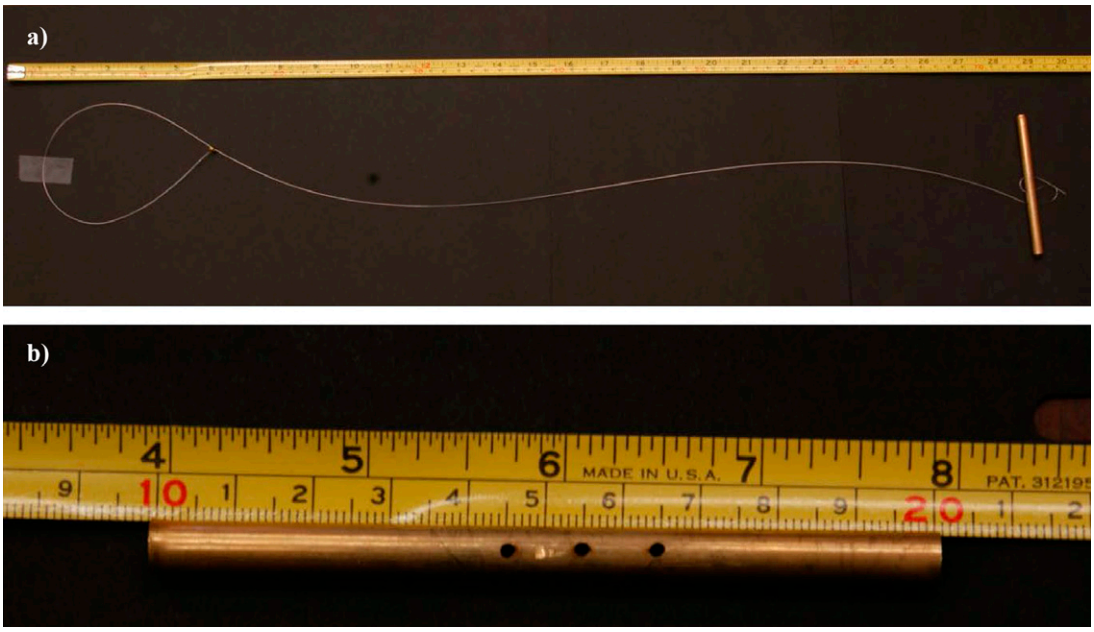

Figure 1. Photographs of the placentome attainment device fully assembled (a) and a close up of the handle depicting placement of perforations (b). See online version for figure in color.

Carroll (1974) and Garcia et al. (1992). Carroll reported the effects of induced (5 mg flutmetasone) to spontaneous parturition in 50 cow–calf pairs, which were found to be comparable (control: 46%; induced: 47.15%). More recently, Garcia et al. (1992) reported that reproductive performance of beef cattle after induced parturition between d 276 to 278 of gestation with and without retained placenta were not statistically different (84 vs. 95%; $P > 0.3$). Using our technique and device, at this point of gestation it appears that investigators can expect a 24% reduction in subsequent pregnancy rate. Considering the minimal therapeutic interventions used during this trial, it may be possible to increase pregnancy rate further by using additional therapies (e.g., therapeutic flush of the uterus with antibiotics/antimycotics, systemic antibiotics) before breeding season. Lastly, differences in endocrine profile between early third trimester [increased progesterone (**P4**)] and the last 30 d of gestation (increased estradiol, increased P4, and increased prolactin) and the absence of a suckling calf are most likely eliciting changes in the uterus (e.g., increased smooth muscle contractions) and/or immune function that would be beneficial to improving subsequent reproductive performance.

The procedure and device provided in this technical report give a detailed description and summary of results from serial placentome collections in cattle after induced abortion. The advantages of this technique are the availability and low cost of the instrument, ease of tissue collection, ability to perform serial collections, and minimal mortality of subjects. Disadvantages include an approximately 30% increase in incidence of open cows after induced abortion and serial tissue collections. These results indicate that intact placentomes representative of tissues in utero can be collected in a serial manner from a large number of animals with minimal risk to the subject and in a cost effective manner. These data will be helpful to those investigators interested in pursuing the physiology behind placental separation and provide veterinary practitioners with an inexpensive instrument to collect tissue samples from cases of suspected placentitis.

LITERATURE CITED

Carroll, E. J. 1974. Induction of parturition in farm animals. J. Anim. Sci. 38:1–9.

Eiler, H., and K. A. Fecteau. 2007. Retained placenta. In: R. S. Youngquist and W. R. Threlfall, editors, Current therapy in large animal theriogenology. 2nd ed. Saunders, St. Louis, MO. p. 345–354.

Garcia, A., A. D. Barth, and R. J. Mapletoft. 1992. The effects of treatment with cloprostenol or dinoprost within one hour of induced parturition on the incidence of retained placenta in cattle. Can. Vet. 33:175–183.

Maj, J. G., and M. Kankofer. 1997. Activity of 72-kDa and 92-kDa matrix metalloproteinases in placental tissues of cows with and without retained fetal membranes. Placenta 18:683–687.

Mee, J. F., F. Buckley, D. Ryan, and P. Dillon. 2009. Pre-breeding ovaro–uterine ultrasonography and its relationship with first service pregnancy rate in seasonal-calving dairy herds. Reprod. Domest. Anim. 44:331–337.

Sharpe, K. L., H. Eiler, and F. M. Hopkins. 1990. Changes in the proportion of type I and type III collagen in the developing and retained bovine placentome. Biol. Reprod. 43:229–235.

Streyl, D., R. Kenngott, N. Herbach, R. Wanke, H. Blum, F. Sinowatz, E. Wolf, H. Zerbe, and S. Bauersachs. 2012. Gene expression profiling of bovine peripartal placentomes: Detection of molecular pathways potentially involved in the release of foetal membranes. Reproduction 143:85–105.