

ASSESSING RECOMBINANT TRYPSIN FOR TREATMENT OF EMBRYOS EXPOSED TO BOVINE HERPESVIRUS-1

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The objective of this study was to determine if treatment with TrypLE™ Select (10×) (Invitrogen, Carlsbad, CA, USA) would prevent IVF-embryo-associated bovine herpesvirus-1 (BHV-1) from infecting uterine tubal cells (UTC). Day seven, IVF, bovine embryos were exposed to 10^{5-7} plaque forming units/mL BHV-1 for 1 h. Following exposure, embryos were trypsin treated according to the IETS protocol with porcine origin trypsin or alternatively with recombinant trypsin (TrypLE™ Select (10×) concentrated or diluted 1:2 for 10 min). Negative and positive controls consisted of non-fertile or degenerated (NFD) embryos washed before or after exposure to BHV-1, respectively. Following treatment, groups of five or single embryos or NFD were placed in IVC with UTC and incubated for 43–48 h at 38.5 °C in 5% CO₂. Following incubation, embryos and corresponding zona pellucida, and UTC were assayed for BHV-1 by plaque assay, and results were compared with positive controls using Chi-square tests. Although all negative control NFD were virus free, BHV-1 was isolated from 100% (8/8) of groups and 32% (9/28) of individual positive control NFD; from 75% (6/8) of groups and 22% (6/27) of individual embryos treated with porcine trypsin; from 20% (1/5) of groups and 24% (4/17) of individual embryos treated with TrypLE™ Select (10×); and from 75% (3/4) of groups and 7% (1/14) of individual embryos treated with TrypLE™ Select (10×) diluted 1:2. Whereas UTC cultured with all negative control NFD were virus free, BHV-1 was isolated from UTC associated with 88% (7/8) of the groups and 50% (14/28) of individual positive control NFD; from UTC associated with 75% (6/8) of the groups and 37% (10/27) of individual embryos treated with porcine trypsin; from UTC associated with 60% (3/5) of the groups and 35% (6/17) of individual embryos treated with TrypLE™ Select (10×); from UTC associated with 75% (3/4) of the groups and 14% (2/14) of individual embryos treated with TrypLE™ Select (10×) diluted 1:2. For inactivating embryo-associated virus, only treatment with TrypLE™ select (10×) of embryos that were subsequently cultured as groups significantly reduced detection of BHV-1. For preventing subsequent infec-

tion of co-cultured UTC, only treatment with TrypLE™ select (10×) diluted 1:2 of embryos that were subsequently cultured as individuals significantly reduced infection of UTC. Thus, although treatment with recombinant trypsin products might reduce the incidence of transmission of BHV-1 associated with IVF embryos, treatment with TrypLE™ Select (10×) for 10 min was not consistently effective in preventing transmission within this in vitro model.

Keywords: Bovine herpesvirus-1; IVF embryos; Trypsin treatment; Recombinant trypsin

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TECHNIQUE FOR RADIOGRAPHIC FLUOROSCOPY EVALUATION OF THE CERVIX AND UTERUS IN ALPACAS

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Hysterosalpingography has been described for evaluation of the uterus and fallopian tubes in humans [1]. A hysterosalpingogram outlines the contour of the uterine cavity and enables assessment of the width of the cervical canal [1]. We describe a technique that we developed for the evaluation of the cervix and uterus in alpacas suspected to have abnormalities. The technique was first standardized on two normal female alpacas before use on clinical cases. All alpacas were at the peak of the follicular wave, as determined by ultrasonography (follicles 8–10 mm in diameter and uterine edema present) at the time of examination. Anesthesia was accomplished by im injection of a combination of ketamine (4 mg/kg), xylazine (0.33 mg/kg) and butorphanol (0.03 mg/kg); the duration of anesthesia was 20–25 min. The female was placed on the radiographic table in left lateral recumbency. The perineal area and vulva were cleaned and disinfected to prevent contamination. An 18-gauge, 30-cm long Foley catheter was introduced vaginally through a sigmoidoscope and placed at the level of the first cervical ring at the external cervical os and held in place by inflating the 30 mL balloon. A total volume of 60 mL of undiluted iodinated positive—contrast medium (Oxilan®) was infused under fluoroscopic guidance and multiple fluoroscopic images were acquired at 2 images/s. This radiographic technique has been performed successfully on four alpacas for diagnosis of cervical abnormalities ($n = 3$) and uterus unicornis ($n = 1$). This radiographic evaluation con-



Fig. 1. Normal cervix.



Fig. 2. Stenotic cervix.



Fig. 3. Spiral cervix.

firmed cervical abnormalities in three alpacas in which cervical catheterization or videoendoscopic examination were impossible.

This imaging technique is a quick and reliable for evaluation of cervical and uterine abnormalities (uterus unicornis, transluminal adhesions) which would otherwise be very difficult to visualize. The technique does not require complete catheterization of the cervix, as long as the female is at the peak of follicular development. Studies are in progress to determine the variation in cervical morphology in normal alpaca females Figs. 1–3. **Keywords:** Alpaca; Hysterosalpingography; Fluoroscopy; Cervical abnormalities

Reference

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RETROSPECTIVE CLINICAL AND CYTOGENETIC STUDY ON OVARIAN HYPOPLASIA IN ALPACAS

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Practicing veterinarians are often required to perform maiden female pre-purchase examinations for fertility. We conducted a retrospective study on 37 cases of ovarian hypoplasia diagnosed in alpacas over the last 8 years in our hospital. Health and breeding history was recorded for all females. After physical examination, a complete reproductive tract evaluation was conducted that included: evaluation of external genitalia, transrectal ultrasonographic evaluation of the uterus and ovaries, vaginal examination and endometrial culture and cytology. A presumptive diagnosis of ovarian hypoplasia was reached when ovaries or normal follicular dynamics were not detected after three consecutive examinations conducted 3 days apart. Confirmation of the diagnosis was made by laparoscopy. Cytogenetic evaluation was performed on 18 females.

The main presenting complaint in the cases studied was repeat breeding (91.9%). Only three females presented with a complaint of refusal to accept the male. The mean age at presentation was 1001 ± 356 days for females with a known birth date. Four females were imported and their ages were not available but were estimated to be between 4 and 4.5 years of age. The mean number of matings per female was 6.4 ± 6.3 (range, 0–28). All females were examined by a veterinarian for infertility prior to presentation and received various treatments. Twenty-one (56.8%) of the females had been treated at least once for uterine infection before presentation. Suspicion of ovarian hypoplasia was a differential posed by the practitioner in only two of the cases. One female had abnormal external genitalia (vulvar aplasia and enlarged clitoris) and one female was taller than normal. Absence of follicular development or inability to visualize the ovaries after three examinations in a 10-day period proved accurate in diagnosing the condition. All females had a flaccid uterus and an easily catheterized cervix.

Cytogenetic evaluation was available for 18 of the females. Ten females had a normal (74, XX) karyotype whereas six others had the following abnormalities: XO, $n = 2$; XXX, $n = 2$; XX/XY, $n = 1$; XO/XX, $n = 1$. An additional two females were described as normal XX with a minute chromosome. Typical gonadal dysgenesis (absence of all follicular stages) was found after