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Evaluation of diagnostic utility, safety considerations, and effect on fertility of transvaginal ultrasound-guided ovarian biopsy in mares

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ABSTRACT

Ultrasound-guided biopsies of corpora lutea have been previously used for research purposes in the mare and cow. However, the health effects and fertility after transvaginal luteal biopsies (TVLB) or transvaginal ovarian biopsies (TVOB) obtained for diagnostic purposes in cases of suspected ovarian tumors have not been previously evaluated in the horse. The aim of this study was to determine the effects on health and fertility of TVLB and TVOB in mares; 53 mares were included in the study (11 control non-biopsied mares, 37 TVLB mares biopsied on one or more of the following Days 8, 10, 12, 15, 21, and 5 TVOB mares with ovarian abnormalities), resulting in a total of 108 TVLB and TVOB cycles and 183 procedures. Mares were divided into Groups 1 to 3 by the number of TVLB per estrous cycle (mare in Group 1 had 1 TVLB procedure, mares in Group 2 had 2 TVLB procedures, and mares in Group 3 had 3 TVLB procedures). Group 4 comprised TVOB mare cycles with ovarian abnormalities (n = 5). Mares were examined to determine day of ovulation (Day 0) and the presence of a corpora lutea using transrectal ultrasonography. Mares were sedated, and an ultrasound-guided transvaginal biopsy was performed of luteal or ovarian tissue. Health effects of TVLB or TVOB were assessed by daily rectal temperatures, appetite, and general demeanor for 72 hours post-procedure, and 3 mares were examined at necropsy. Fertility was not significantly different in control and TVLB Groups 1 to 3 (P = 0.7648) and in the first or subsequent cycles where the ovulation was from an ovary that had a previous TVLB (P = 0.7147). A TVLB on Day 8 post-ovulation may induce an early return to estrus. In conclusion, the TVLB or TVOB procedure had no effect on health and fertility in this study if the procedure was correctly performed with good technique. Because of the low number of cycles (n = 37), the fertility data should be interpreted with caution. The TVOB may be applied in the diagnosis of mares with ovarian abnormalities, and no adverse health effects were associated with TVOB of mares with granulosa theca cell tumor.

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1. Introduction

Ultrasound-guided biopsies of corpora lutea (CL) for research purposes in the mare and cow have been described by Slough et al. and Aerts et al. [1,2]. Before the description of transvaginal ultrasound-guided CL biopsies, a related technique was described for transvaginal ultrasound-guided aspiration of follicles in the mare dating back to 1988 [3–7]. The transvaginal oocyte recovery is performed by puncture and aspiration of ovarian follicles. It has been determined that repeated follicular aspirations in mares can cause fibrosis in the ovarian stroma but have no adverse effects on fertility [8–11]. However, the information on the health and fertility effects of transvaginal luteal biopsies (TVLB) or transvaginal ovarian biopsies (TVOB) had not been reported. Complications that may arise from transvaginal ovarian manipulations include hemorrhage or the inadvertent introduction of bacteria causing an ovarian abscess [10].

The nature of ovarian abnormalities is determined using a variety of modalities including transrectal palpation, ultrasonographic examination, advanced imaging techniques (magnetic resonance imaging and computed tomography), hormone analysis, and histopathologic means. Ovarian tissue may be obtained for analyses through ovarian biopsy or ovariectomy. Laparoscopic visualization and examination of the equine ovary have been reported for diagnostic purposes when ovarian abnormalities, such as tumors, are suspected but not confirmed [12–14]. The presence of ovarian tumors in mares is associated with reduced fertility [12–15]. Neoplasia of the ovary includes gonadostromal tumors (granulosa theca cell tumors [GTCT]), epithelial tumors (cystadenomas and adenocarcinomas), and germ cell tumors (teratomas and dysgerminomas) [14].

The GTCT is the most common ovarian tumor in the mare and has been reported to have a classic ultrasonographic appearance referred to as a "cluster of grapes," along with a small inactive contralateral ovary [12–15]. The GTCT may, however, be bilateral, and unusual echotextural features have been reported [16–18]. Epithelial tumors in mares may present with a relatively normal echotextural appearance. Other differentials for an ovary with an unusual or abnormal echotexture include persistent anovulatory follicles, ovarian hematomas, epithelial inclusion cysts, and multiple supplemental CLs found in pregnancy [15].

Hormonal assays are used to assist in the diagnosis of ovarian abnormalities. Circulating levels of testosterone, inhibin, and anti-müllerian hormone have been used as tumor biomarkers for GTCT and androgen levels in mares with ovarian cystadenomas, but most other ovarian tumors do not produce reliable biomarkers [19–22]. Elevated levels of these hormonal serum biomarkers may indicate that abnormal ovarian tissue is present but do not locate the source and would not, therefore, differentiate unilaterally from bilaterally affected mares. The size of the mare and the deep abdominal position of the ovaries mean that diagnostic modalities, such as magnetic resonance imaging and computed tomography, are problematic for use in determining the nature of an ovarian abnormality. Surgical removal of the suspected abnormal ovary is often performed as it usually provides a definitive diagnosis and may be curative [15]. There are select cases where an ovarian biopsy would be useful in determining when it is suspected that both ovaries are neoplastic or to determine the nature of an ovarian abnormality before invasive and irreversible ovariectomy. Criteria for the use of a TVOB might include abnormal ovarian consistency, inconclusive hormonal findings, questionable or abnormal ultrasonographic echotexture of the ovary, undiagnosed behavioral or estrous cycle irregularities, difficulty in accessing laboratories that measure biomarkers, and investigation of possible bilateral tumor involvement.

The health and fertility of mares experiencing TVLB or TVOB has not been reported, and the TVOB of neoplastic ovarian tissue has not been described in the mare. Therefore, the first hypothesis was that there would be no health or fertility effects in healthy mares and mares with ovarian abnormalities after the TVLB or TVOB procedures. It was hypothesized that the TVOB of neoplastic ovarian tissue may be used to obtain samples sufficient for making a diagnosis without adverse effects.

2. Materials and methods

2.1. Animals

The experimental protocol was approved by the Institutional Animal Care Committee of the University of Saskatchewan. Mares (n = 53) of Quarter Horse, Paint, Warmblood, Thoroughbred, and Arabian breeds with a mean age of 12 years and ranging in age from 4 to 28 years were followed throughout multiple estrous cycles before and after ovarian procedures using transrectal palpation and ultrasonography of the reproductive tract. Mares in this study had 1 to 5 TVLB cycles per year. After a TVLB cycle, the majority of mares were administered 5 mg prostaglandin F_2-alpha (PG) (Lutalyse; Zoetis, Kirland, Quebec, Canada) and given a rest cycle before being enrolled in another TVLB cycle. Interestrus intervals were calculated in non–PG-treated mares. The TVLB samples collected in this study were obtained as part of another research study.

The numbers of mares studied per year in the 3-year period by type of procedure are listed in Table 1. In 2012, there were 21 mares included in the study: 4 healthy control (non-biopsied) mares, 13 healthy TVLB mares, and 4 mares (TVOB) with ovarian abnormalities. In 2013, there were 18 mares included in the study: 3 healthy control mares, 14 healthy TVLB mares, and 1 TVOB mare with an ovarian abnormality. Five mares (1 control, 4 TVLB mares, and 1 TVOB mare with an ovarian abnormality) were used in both years. In 2014, there were 14 mares included in the study: 4 healthy control mares and 10 TVLB mares included in the study. Over the 3-year period, there were a total of control (n = 11), TVLB (n = 37), and TVOB (n = 5) mares studied.

In the population of 53 mares, multiple cycles were studied (n = 108) and were divided into groups as follows: control; Group 1, 1 TVLB per cycle; Group 2, 2 TVLB per cycle; and Group 3, 4 TVLB per cycle; and Group 4 mares with suspected ovarian abnormalities where a TVOB was performed. Group 1 (n = 43 cycles) mares were biopsied on