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# Luteal function induced by transvaginal ultrasonic-guided follicular aspiration in mares

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ABSTRACT

Ultrasonic-guided transvaginal follicle aspiration was performed in 58 crossbreed mares in order to determine whether aspiration of various dominant follicle diameters resulted in luteal tissue capable of producing progesterone ($P_4$). The mares were randomly assigned to three groups according to follicular diameter (25–29 mm; 30–35 mm and >35 mm). Mares that had ovulations naturally served as controls. The serum progesterone ($P_4$) concentrations in the aspirated mares were greater ($P < 0.0001$; $r^2 = 0.6687$; CV = 21.52) in mares with natural ovulation compared to mares with aspirated follicles regardless of groups. Serum $P_4$ concentration in aspired mares with follicular diameter of 25–29 mm declined 0.365 ng/ml/day ($P = 0.0065$) from the day of aspiration (D0) up to D8. In mares with follicle diameter of 30–35 mm, serum $P_4$ concentration increased (0.258 ng/ml/day; $P = 0.001$), as well as in the mares with follicles >35 mm diameter (0.481 ng/ml/day; $P < 0.0001$), and in mares with natural ovulation (1.236 ng/ml/day; $P < 0.0001$). Out of the 25 mares with follicular aspirations that formed Corpora hemorragica ($P_4$ >1 ng/ml), 23 (92%) had greater (>2 ng/ml) serum $P_4$ concentrations on Day 8 after aspiration. Of these 23 mares, 75% were in the 25–29 mm group, 9/10 (90%) in the 30–35 mm group, and 11/11 (100%) of the mares in the >35 mm follicular diameter group had luteinization ($P_4$ >2 ng/ml). These results suggest that a functional Corpus luteum can be induced in mares using follicular aspiration and that a minimum 35 mm follicular diameter is needed to reach a progesterone serum concentration compatible with that of a Corpus luteum produced by natural ovulation.


## 1. Introduction

Follicular aspiration has been used to study the follicular dynamics in horses and cattle without the interference of exogenous steroids (Gastal et al., 1997; Ginther et al., 2003), both for induction of follicular wave emergence synchronization of time of ovulation (Bergfelt et al., 1994, 1997; Amiridis et al., 2006; Lima et al., 2007).

Studies in mares (Hinrichs et al., 1991; Bracher et al., 1993; Goudet et al., 1997; Meintjes et al., 1997; Bogh et al., 2000; Hayna et al., 2004) indicated luteal tissue can be formed after follicular aspiration. Luteal function is important to ensure adequate uterine environment for the embryo in pregnant mares, and to ensure a regular estrous cycle in non-pregnant mares. In an embryo transfer program, inducing ovulation in mares with various size follicles would greatly facilitate synchronization of donor and recipient mares.

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Procedures conducted in humans and primates indicated luteal dysfunction after follicular aspiration of preovulatory follicles (Kreitmann et al., 1981) and failures in human *in vitro* fertilization were related to lesser maternal progesterone concentrations (Kreitmann et al., 1981).

Early luteinization of preovulatory follicles after follicular aspiration in mares has been described by Hinrichs et al. (1991), Bracher et al. (1993), Bogh et al. (2000) and Hayna et al. (2004). However, luteinization in aspirated follicles was less common when mares were in dioestrus (Bogh et al., 2000) or during the transitional period (Alvarenga et al., 1999). Although studies reported that aspiration of a dominant follicle in mares might promote the formation of a morphological and biologically active Corpus luteum, the luteinization process after aspiration still warrants an enhanced understanding. It has been suggested that the follicular diameter of the aspirated follicle may influence the subsequent Corpus luteum formation (Hinrichs et al., 1991; Bogh et al., 2000; Hayna et al., 2004).

The present study aimed to determine the effect of follicular aspiration on luteinization in mares with aspirated follicles between 25 and 29 mm, 30 and 35 mm and >35 mm in diameter. Progesterone concentration in these mares was compared to blood progesterone in mares with natural ovulation.

## 2. Materials and methods

### 2.1. Experimental animals

Crossbreed mares ($n = 59$) aged between 5 and 22 years with body weight ranging from 350 to 500 kg were used. All mares were kept on pasture without supplementation.

The research was conducted during the reproductive seasons of 2004/2005, 2005/2006, 2006/2007 and 2007/2008 between October and February, in the town of Uruguaiana-RS (latitude 30°12′26″S and longitude 57°33′17″W), southern Brazil.

The mares were examined daily by palpation per rectum to assess the uterine size, consistency, symmetry and to observe follicular dynamics. The presence and diameter of follicular structures were observed through ultrasonic with 5 MHz linear rectal transducer. Mares with follicular diameter equal to or larger than 25 mm (independent of the stage of the estrous cycle) were assigned to treatment groups according to the follicular diameter: 25–29 mm; 30–35 mm and >35 mm. All mares had their follicles aspirated as soon as they were identified and assigned to a treatment group. Mares with natural ovulation served as controls.

### 2.2. Ovarian follicular aspiration

Before follicular aspiration, mares were restrained in stocks and submitted to epidural anesthesia using 5 ml of lidocaine 2% without epinephrine associated with previous sedation with xylazine 10% (0.45 mg/kg, iv) and acepromazine (0.05 mg/kg iv). The tail of the mare was wrapped, fecal material was removed from the rectum, and the vulva and perineal area were cleaned using a dry paper towel.

The follicular aspiration procedure was performed with an ultrasonic device (Pie Date 240 Parus Vet, England). The system was equipped with a 5 MHz sector transducer, a stainless steel aspiration guide, a vacuum pump (COOK-VMAR500, Australia), a silica tube (COOK-V-OPAL-1800, Australia), and a single lumen 16 gauge needle. The vacuum pressure was adjusted to 150 mmHg.

The aspiration guide was inserted into the vagina and placed in the vagina fornix moving the transducer toward the ovary to be aspirated. The follicles were lined up to the ultrasound-guided needle by manual manipulation of the ovary per rectum. All follicles larger than 10 mm were randomly aspirated and no search for oocytes in the recovered follicular fluid was performed. The ovaries were monitored by rectal palpation and transrectal ultrasonography daily until the eighth day after follicle aspiration. The procedure was performed to observe the development of new follicles. The mares with natural ovulation (control) were monitored daily in the same way until the sixth day after ovulation.

The serum progesterone concentration was used to identify luteinization rates (mares with $P_4$ concentrations >1 ng/ml/total of aspirated mares in each group). Increased $P_4$ concentrations >1 ng/ml was considered as luteinization.

### 2.3. Blood samples

Blood samples were daily collected through jugular venipuncture into non-heparinized tubes, beginning right before aspiration (D0) until the eighth day thereafter (D8). The day of ovulation for control mares was identified by ultrasonography and considered day zero (D0). Blood samples of these mares were colleted daily until D6, and stored at room temperature in 10 ml tubes until the retraction of the blood clot. The serum samples were stored at −20 °C for progesterone radioimmuneassay (RIA) evaluation. These samples were analyzed at B.E.T. Laboratories, University of Kentucky Coldstream Research Campus 1501 Lexington, KY, USA.

### 2.4. Statistical analysis

Statistical program SAS was used (SAS Institute, Cary, NC, 1998). Data were analyzed by ANOVA to verify the $P_4$ production from each aspirated follicle related to the follicular diameter, the day after aspiration and the follicular diameter compared with day interaction. The Tukey test was used to compare the average concentration of $P_4$ between treatment groups related to the day after aspiration. Analysis of progesterone concentration values required the addition of correction factor (+1.5), and square root extraction for testing normality of data. The GLM procedure was performed to compare the progesterone concentration by follicular group on each data, and the average time (days) so that progesterone $P_4$ production reached concentrations >1 ng/ml. Data were submitted to linear regression analysis to establish the curve of progesterone using the Pearson correlation coefficient. The number of luteinized follicles was analyzed using the