



# Influence of transvaginal ultrasound-guided follicular punctures in the mare on heart rate, respiratory rate, facial expression changes, and salivary cortisol as pain scoring



Rodrigo Diego<sup>a</sup>, Cécile Douet<sup>a</sup>, Fabrice Reigner<sup>b</sup>, Thierry Blard<sup>b</sup>,  
Juliette Cognié<sup>a</sup>, Stefan Deleuze<sup>c</sup>, Ghylène Goudet<sup>a,\*</sup>

<sup>a</sup> PRC, INRA, CNRS, IFCE, Université de Tours, Nouzilly, France

<sup>b</sup> PAO, INRA, Nouzilly, France

<sup>c</sup> Faculté de Médecine vétérinaire, Département des Sciences Cliniques-Clinique Equine, Université de Liège, Liège, Belgium

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## ABSTRACT

Transvaginal ultrasound-guided follicular punctures are widely used in the mare for diagnosis, research, and commercial applications. The objective of our study was to determine their influence on pain, stress, and well-being in the mare, by evaluating heart rate, breath rate, facial expression changes, and salivary cortisol before, during, and after puncture. For this experiment, 21 pony mares were used. Transvaginal ultrasound-guided aspirations were performed on 11 mares. After injections for sedation, analgesia, and antispasmodia, the follicles from both ovaries were aspirated with a needle introduced through the vagina wall into the ovary. In the control group, 10 mares underwent similar treatments and injections, but no follicular aspiration. Along the session, heart rate and breath rate were evaluated by a trained veterinarian, ears position, eyelid closure, and contraction of facial muscles were evaluated, and salivary samples were taken for evaluation of cortisol concentration. A significant relaxation was observed after sedative injection in the punctured and control mares, according to ear position, eyelid closure, and contraction of facial muscles, but no difference between punctured and control animals was recorded. No significant modification of salivary cortisol concentration during puncture and no difference between punctured and control mares at any time were observed. No significant modification of the breath rate was observed along the procedure for the punctured and the control mares. Heart rate increased significantly but transiently when the needle was introduced in the ovary and was significantly higher at that time for the punctured mares than that for control mares. None of the other investigated parameters were affected at that time, suggesting discomfort is minimal and transient. Improving analgesia, e.g., through a multimodal approach, during that possibly more sensitive step could be recommended. The evaluation of facial expression changes and heart rate is easy-to-use and accurate tools to evaluate pain and well-being of the mare.

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## 1. Introduction

Transvaginal ultrasound-guided follicle aspiration was initially developed for oocyte recovery in women in association with *in vitro* fertilization (IVF) [1,2]. The technique was then adapted to the cow [3] and is nowadays conducted routinely in women and cows for oocyte collection

\* Corresponding author. Tel.: +33 (0) 2 47 42 79 41; fax: +33 (0) 2 47 42 77 43.

E-mail address: [ghylene.goudet@tours.inra.fr](mailto:ghylene.goudet@tours.inra.fr) (G. Goudet).

for IVF. In the mare, transvaginal ultrasound-guided follicular puncture (ovum pickup, OPU) has been developed in the early 90s [4–6] and is widely used for diagnosis [7,8] and research purposes [9–22]. Moreover, commercial applications have been developed [23–26]. As each OPU session involves multiple needle punctures into the vagina wall, peritoneum, and the ovaries and because the same mare may be used again for later sessions, identification and alleviation of pain is essential for animal welfare. Previous studies have analyzed the effect of repeated transvaginal aspiration of immature follicles on mare health and ovarian function and morphology [27,28] or fertility [21,29]. However, to our knowledge, the effect of transvaginal follicle aspiration on stress, pain, and well-being has never been studied in the mare. Our hypothesis was that transvaginal aspiration of follicles may induce stress and pain that could affect well-being. Therefore, our aim was to determine the influence of transvaginal ultrasound-guided follicle aspiration on stress, pain, and well-being in the mare.

Stress induces two different physiological responses: a rapid reaction characterized by an increase in catecholamine levels, which in turn increases heart rate, and a long-term adjustment response, which results in an increased glucocorticoid level, cortisol being the dominant glucocorticoid in horse plasma [30]. For example, during transport, which includes multifactorial stressors, increased salivary cortisol concentrations and changes in heart rate have been observed [31,32]. A positive correlation between equine serum and salivary cortisol concentrations has been established [33]. Moreover, serum cortisol includes both free and bound inactive fractions, whereas salivary cortisol represents a part of the free cortisol fraction, which is the biologically active form [33]. The analysis of salivary cortisol is a validated noninvasive technique avoiding stress induced by repeated intravenous blood sampling [33].

As in other animal species, pain in horses is difficult to assess, but pain-related behaviors have been identified [34,35]. However, many of these behaviors have been observed in response to severely painful conditions and may not be induced by mild to moderate pain. Facial expressions are used to assess pain in humans [36,37], mice [38], rats [39], and rabbits [40]. Recently, a standardized pain scale based on facial expressions in horses (Horse Grimace Scale) has been developed and validated [41]. The Horse Grimace Scale offers an effective and practical method of identifying painful conditions.

Our objective was to determine the influence of transvaginal ultrasound-guided follicular punctures on pain, stress, and well-being in the mare, by evaluating heart rate, respiratory rate, facial expression changes, and salivary cortisol before, during, and after puncture.

## 2. Materials and methods

All procedures on animals were conducted in accordance with the guidelines for the care and use of laboratory animals issued by the French Ministry of Agriculture and with the approval of the ethical review committee (Comité d’Ethique en Expérimentation Animale Val de Loire) under number 02701.02.

### 2.1. Transvaginal ultrasound-guided follicular punctures

For this experiment, 21 adult cyclic pony mares from our experimental study, from 3 to 21 year old and from 265 to 390 kg were used in May and June. The mares were familiar with the staff, the OPU premises, and the material used. Their ovarian activity was assessed by routine rectal ultrasonography, using a 7.5-MHz transrectal probe (Aloka, Wallingford, USA) for choosing mares with several follicles from 5 to 25 mm.

Transvaginal ultrasound-guided aspirations were performed on 11 mares (ovum pickup, OPU group). Mares entered the puncture room and were restrained in stocks. They received a first injection of detomidine (Medesedan, 10 µg/kg intravenously, detomidine chlorhydrate; Centravet, Plancoet, France) for sedation and analgesia. After evacuation of feces from the rectum, the perineal area was cleaned with povidone-iodine scrub and a urinary probe was introduced in the bladder. Just before the OPU procedure, mares were injected detomidine (Medesedan, 15 µg/kg intravenously), butorphanol (Dolorex, 10 µg/kg intravenously, butorphanol tartrate; Centravet) for analgesia, and butylscopolamine (Estocelan, 0.2-mg/kg butylbromide scopolamine and 100-mg/kg sodium metamizole [dipyrone], intravenously, Centravet) for analgesia and antispasmodia, to induce smooth muscle relaxation. Ultrasound-guided transvaginal follicular aspiration was performed following a routine procedure in our laboratory as previously described [10,42]. Briefly, the ultrasound transducer was introduced into the vagina, whereas the ovary was manipulated per rectum to position the follicles to be aspirated in line with the needle-guide on the screen. Then, the needle was introduced through the vaginal wall into the follicle. The content of the follicle was aspirated with a double-lumen needle (length 700 mm, outer diameter 2.3 mm, internal diameter 1.35 mm; Casmed, Cheam, Surrey, England) by use of a vacuum pump at 150 mm Hg. Flushes of the follicle with heparinized PBS (phosphate-buffered saline, Dulbecco A; Oxoid, Basingstoke, Hampshire, England and heparin, Choay, Sanofi Aventis 5000 IU/mL) were repeated 10 times. The procedure was repeated for all follicles above 10 mm on both ovaries. All collected fluids were examined for oocyte recovery. Mares received an injection of antibiotics (Depocilline, 15000-IU/kg benzylpenicillin, intramuscularly, Intervet, Beaucauze, France) at the end of the puncture session.

In the control group, 10 mares underwent similar treatments (emptying of the rectum, cleaning of the perineum, and urinary probe) and similar injections (10-µg/kg detomidine, 10-µg/kg butorphanol, 0.2-mg/kg butylbromide scopolamine, and 100-mg/kg sodium metamizole) but without introduction of the ultrasound transducer into the vagina or subsequent follicular puncture. No antibiotics were injected.

Each day, after ovarian-activity assessment, mares with the largest number of follicles joined the OPU group, whereas those with fewer follicles were used as controls.

### 2.2. Heart rate, respiratory rate, and facial expression changes

Heart and respiratory rate were evaluated by a trained veterinarian using a stethoscope. Ears position (turned

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