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Original Research

Assessment of Prolactin and Quantitative Milk Production After Induction of Lactation in Barren Jennies (*Equus asinus*): A Pilot Study

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ABSTRACT

The secretion of circulating prolactin levels in response to sulpiride treatment was evaluated in eight mature cyclic barren jennies divided into two groups on the basis of different treatments. The experimental group A was submitted to the sulpiride treatment twice a day for nine consecutive days, up to 14 days after ovulation, and the control group B was submitted to the equivalent saline placebo administration. Milking was started at 8 days after ovulation, at 3 days after the beginning of sulpiride treatment, to 28 days after ovulation, and performed five times per day between 09:00 AM and 11:00 PM by hand; the oxytocin treatment was performed (5 IU/head per intramuscular injection) 5 minutes before milking, to facilitate the udder contraction. Blood samples were collected in baseline conditions (the day before the onset of the treatments) from day 0, during all treatment periods, until day 28 after ovulation, from the jugular vein from the each subject, twice a day, at 08:00 AM and at 08:00 PM. Both pluriparous experimental ($P < .0005$) and control ($P < .005$) jennies and nulliparous experimental ($P < .005$) and control ($P < .0005$) jennies showed higher morning prolactin (PRL) concentrations than afternoon values. Nulliparous experimental jennies showed higher morning ($P < .0001$) and afternoon ($P < .0001$) PRL values than nulliparous control jennies. Significant and negative correlation between PRL concentrations and milk production ($r = -0.67$) was observed only in experimental pluriparous jennies. The present pilot study demonstrates the PRL profiles during and after induction of lactation in barren pluriparous and nulliparous Ragusana jennies.

1. Introduction

Prolactin (PRL) is the main hormone involved in inducing lactation in equines, and its sudden increase in healthy gestation could be the trigger for the onset of lactation [1–3]. Prolactin secretory pattern is also involved in response to physical [4] and emotional [5] stimuli, with a temporal relationship [6,7] in equines, and significant changes in PRL levels have been observed in response to excitement and stress in stallions and geldings [5]. Therefore, the evaluation of PRL receptor as a candidate gene for male fertility in horses was reported [8], and specific receptors in the mature corpus luteum for equine PRL have been reported [9], with seasonal variation in the inhibition of PRL secretion by dopaminergic systems [10,11]. Dopamine antagonists such as sulpiride are used experimentally to induce cyclicity during winter transition in mares [12–14], with an increase

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of PRL secretion [13,15,16] and milk secretion after treatment [17–19]. It has been recognized that milk production after pharmaceutical induction of lactation in mares can offer practical and economical advantages [3,17,20]. Though, in mares, the changes in plasma PRL in response to injection of sulpiride were greater than for thyrotropin-releasing hormone treatment [21].

Donkeys are endocrinologically and metabolically different from horses. Although jennies have shown many physiological reproductive similarities with mares [20–24], several specific differences have been observed [25–27]. Jennies are increasing in popularity as a result of the importance of their milk for nutritional and extranutritional properties. Although numerous studies have been carried out on the composition of broodmare and donkey milk [28], few studies have been performed on the physiological changes in relation to handling methods and different breeds [29–32]. Nevertheless, the effect of pharmacologic agents on circulating PRL concentrations in milking jennies has not been studied and no data are available.

The purpose of the present preliminary study was to characterize the physiological patterns of PRL response in barren cyclic jennies submitted to sulpiride treatment and hand milking, evaluating the differences between experimental treated subjects (group A) and control untreated subjects (group B).

2. Materials and Methods

2.1. Jennies

The study comprised eight healthy mature cyclic Ragusana barren jennies (four pluriparous aged 6 years and four nulliparous aged 3 years), weighing 325 ± 25 kg. Four jennies were sampled in 2012, and four jennies were sampled in 2013. The breeding season of jennies in Sicily takes place from February to July. The subjects were studied in March and April and were divided into two groups on the basis of different treatments. The animals were maintained under natural photoperiod and ambient temperature. The jennies were randomly assigned to one of two groups based on different treatments: group A was designated as the experimental group and included two pluriparous and two nulliparous jennies; group B was designated as the control group and included two pluriparous and two nulliparous jennies. Jennies stabled on Animal Reproduction Centre and Assisted Conception of Department of Veterinary Sciences of Messina, Sicily, Italy (38° 13′ 19″ 92 N latitude; 15° 14′ 20″ 76 E longitude), 100 m at sea level. All jennies had free access to pasture during the day and were individually fed 3.5 kg of a grain supplement, straw, and vetch hay twice a day. The composition of the grain supplement was the same for both groups, to minimize the effect of diet differences. Water and mineral supplements were always available *ad libitum*. The animals were kept in paddocks during milking. Milking routines were kept constant, being performed by the same person for each group.

After insertion of a 14-gauge catheter in the left jugular vein of each jenny, they were allowed to stand quietly a minimum of 1 hour before blood sampling. Blood samples were collected from, and treatment injections were administered through, the jugular catheters. The 10 samples of plasma for each mare on each treatment day were assessed for PRL concentration.

Table 1
Protocol for inducing synchronization in barren jennies by hormonal treatment.

Days	Hormonal Treatments Before Synchronization
0–7	Altrenogest (Regumate; 0.044 mg/kg BW per os)
8	Prostaglandins, $PGF_{2\alpha}$ (Gabbrostim; 3 mg/subject IM)
11	hCG (Corulon; 2,500 IU/subject IV)

Abbreviations: BW, body weight; hCG, human chorionic gonadotropin; IM, intramuscular, IV, intravenous; per os, oral administration.

Ovaries of each jenny were daily assessed by ultrasonography on day 0 and every day, using a portable ultrasound scanner (Aquila-Esaote-PieMedical, Genoa, Italy) equipped with endocavitary linear multisound (6–0, 8–0 MHz), and the size and number of follicles were recorded for each ovary. Before the beginning of treatment, to assure that the mares of both groups were in the follicular phase, their cyclicity was synchronized by the administration of altrenogest (Regumate; 0.044 mg/kg body weight, oral administration) once a day, for 7 consecutive days; at the 8th day, the subjects were submitted to the administration of prostaglandins, $PGF_{2\alpha}$ (Gabbrostim; 3 mg/subject per intramuscular injection). After a follicle of >35 mm in diameter was detected, the ovaries of jennies were scanned daily until ovulation that was induced using human chorionic gonadotropin (Corulon; 2,500 IU/subject intravenously) at 11 days (4 days after the $PGF_{2\alpha}$ treatment) (Table 1). After the synchronization of ovulation, the experimental group A was submitted to the single $PGF_{2\alpha}$ administration at 6 days after ovulation (3 mg/subject per intramuscular injection), plus sulpiride treatment (Championyl; Sanofi-Synthelabo S.P.A., Milan, Italy; 1 mg/kg body weight) twice a day for 9 consecutive days (8 AM and 8 PM), until day 14 after ovulation (Table 2). The site of injection was systematically changed to avoid local side effects. Sulpiride was selected for use as a secretagogue in the present experiment according to its use in previous studies for the induction of lactation in mares. The control group B simultaneously received the equivalent saline placebo administration, respecting the same treatment's time of group A. The pluriparous and nulliparous jennies' udder of group A was daily examined, by taking into account its size, shape, and turgidity. Milking began 3 days after the onset of sulpiride treatment, at 8 days after

Table 2
Protocol for inducing lactation in barren jennies by hormonal and pharmacologic treatments.

Postovulation Days	Hormonal Plus Pharmacologic and Placebo Treatments After Synchronization	
	Group A: Experimental Group	Group B: Control Group
6	Prostaglandins $PGF_{2\alpha}$ (Gabbrostim; 3 mg/subject IM)	Saline placebo solution
6–14	Sulpiride (Championyl; 1 mg/kg BW IM)	Saline placebo solution

Abbreviations: BW, body weight; IM, intramuscular.