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The effect of isosorbide dinitrate on uterine and ovarian blood flow in cycling and early pregnant mares: A pilot study

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ABSTRACT

Poor uterine perfusion has been proposed as a cause of infertility in mares. The objective of this study was to investigate the effect of isosorbide dinitrate (ISDN), a nitric oxide donor, on uterine and ovarian blood flow resistance during diestrus and early pregnancy in mares. Six Trotter mares, aged 7 to 14 years, were examined daily during the first 11 days of three diestrous periods, and five of those mares were also examined during the first 11 days of two pregnancies. Six mares randomly received a placebo, a low dose (30 mg, ISDN30), or a high dose of ISDN (60 mg, ISDN60) through three nonconsecutive cycles. The treatments were administered orally, every 12 hours from Day 1 to 11 of the cycle (Day 0 = ovulation). Five of the 6 mares received a placebo or 60 mg of ISDN orally every 12 hours from Day 1 to 11 of pregnancy. The mares were short cycled on Day 12 of each trial. Transrectal color Doppler was used to determine blood flow resistance semiquantitatively and expressed as pulsatility index. Mean pulsatility index of both uterine arteries combined and of the dominant (ipsilateral to the CL) ovarian artery was lower (treatment effects: $P \leq 0.01$; time effects: $P \leq 0.002$) in mares receiving 30 mg or 60 mg of ISDN compared with placebo-treated mares. Blood flow resistance in the dominant ovarian artery was lower in ISDN-treated pregnant mares than in placebo-treated pregnant and cycling mares (treatment effect: $P = 0.04$; time effect: $P = 0.003$). Isosorbide dinitrate increases uterine and ovarian perfusion in cycling mares and ovarian perfusion in early pregnant mares. Further studies are needed to investigate these effects in relation to fertility of the mare.

1. Introduction

Doppler sonographic studies have shown that uterine and ovarian blood flow varies with the hormonal status of the mare [1]. Sufficient blood flow is required for maintaining organ function. In the reproductive tract, adequate blood supply is essential for hormonal signaling, uterine contractility, placentation, and fetoendometrial interactions [2].

An early Doppler ultrasonographic study of uterine blood flow in mares proposed that fertility of mares might be related to uterine blood flow [3,4]. The vascular layer of the uterus is located between the circular and longitudinal myometrial layers [5]. It has been hypothesized that elastosis of myometrial blood vessels and degeneration of large vessels in the vascular layer of the uterus indicate reduced uterine blood flow and are related to compromised uterine contractility in aged, pluriparous, subfertile or infertile mares [6]. Impaired uterine contractility in turn may lead to persistent mating-induced endometritis and subsequent subfertility in mares [7]. Local vascular dysfunction has been suspected as a cause of endometrial cysts in mares; pulse wave velocity increased in the adjacent mesometrium, and blood flow volume decreased in the endometrium near the cyst [8]. In women, poor uterine perfusion has been suspected to cause infertility [9–11]. To improve uterine perfusion and the rate of implantation in women

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with poor uterine circulation and fertility problems, reproductive steroids [9], anticoagulants, or vasodilator drugs have been used therapeutically [12,13]. Acetylsalicylic acid, an anticoagulant, and the angiotensin-converting enzyme inhibitor (captopril) improved uterine and ovarian perfusion in cycling mares [14], whereas the anticoagulant pentoxifylline did not affect uterine blood flow [15] and exogenously administered estradiol benzoate and altrenogest reduced uterine perfusion [16].

Perfusion of an organ may be improved through vasodilation. Vasodilation results from relaxation of smooth muscle cells within the vessel walls. Nitric oxide (NO) released from the endothelium or generated by the metabolism of NO donors induces membrane hyperpolarization and stimulates activity of soluble guanylyl cyclase, which increases the synthesis of guanosine 3′,5′-cyclic monophosphate. The production of 3′,5′-cyclic monophosphate triggers a cascade of events that reduces cytosolic Ca^{2+} concentration and promotes relaxation of vascular smooth muscle [17,18]. In contrast to previously tested substances, NO has the advantage of being a physiological component in the regulation of blood flow in the genital tract [19]. The drug isosorbide dinitrate (ISDN) is an organic nitrate and acts as an NO donor [20].

The objective of the present study was to determine the effects of a vasodilator (ISDN) on uterine and ovarian perfusion during the estrous cycle and early pregnancy in mares.

2. Materials and methods

2.1. Animals

Six healthy, cycling Trotter mares, 7 to 14 years of age, were used. The two oldest mares were 14 (A) and 13 years (B) of age and pluriparous, and the remaining four mares were 11, 9, 8, and 7 years (C–F) of age and nulliparous. The study was conducted between August and November and April and May in the Northern Hemisphere. The mares were exposed to natural light and kept in a barn at night and in a paddock during the day.

The study protocol was approved by the Animal Ethics Committee of the Upper Bavarian District Government (Department of Environment, Health and Consumer Protection).

2.2. Study design

At the start of the study, the mares were given an intramuscular injection of 500 μg of the $PGF_{2\alpha}$ analogue cloprostenol (Estrumate, MSD; Unterschleissheim, Germany), and the subsequent ovulation was considered the beginning of the first cycle. All ensuing cycles started with ovulation that was preceded by prostaglandin-induced luteolysis. All mares were examined daily between 12 PM and 2 PM from Day 0 (day of ovulation) to Day 11 during at least five cycles. The treatments consist in the administration of 60 mg of lactose (placebo), 30 mg of ISDN (low dose), or 60 mg of ISDN (high dose; ISDN-Stada; STADApharm, Bad Vilbel, Germany). Each treatment was randomly assigned to one of the first three study cycles every 12 hours from Day 1 to 11 of the cycle (Day 0 = ovulation). On day 12 of each cycle, luteolysis was induced by intramuscular injection of cloprostenol. All mares were inseminated on their fourth and fifth study cycles. Two different treatments consisting in the administration of either 60 mg of lactose (placebo) or 60 mg of ISDN (high dose) from Day 1 to 11 were randomly assigned to one of the two cycles. The mares were examined for pregnancy on Day 12.

Pregnant and nonpregnant mares were short cycled with $PGF_{2\alpha}$ and rebred with the goal of collecting data from two pregnancies per mare during the study. There was one untreated estrous cycle between each treated cycle or treated pregnancy to reduce the probability of a treatment order effect. In all cycles, uterine swabs were taken on the first day of behavioral estrus for bacteriologic and cytologic examination.

The mares were examined using B-mode ultrasonography daily during estrus until ovulation (Day 0) was detected.

2.3. Treatments

The treatments consist of 60 mg of lactose (placebo), 30 mg of ISDN (low dose), or 60 mg of ISDN (higher dose) and were administered every 12 hours, 6 AM and 6 PM, from Day 1 (Day 0 = ovulation) to Day 11 of the cycle.

The placebo and the ISDN were available as tablets and were dissolved in water, added to bran and grain and fed to the mares.

For the second part of the study, all the mares were artificially inseminated and either 60 mg of lactose (placebo) or 60 mg of ISDN was administered every 12 hours, 6 AM and 6 PM, from Day 1 (Day 0 = ovulation) to Day 11 of the cycle.

Pregnancies were referred to as placebo or ISDN60 pregnancies.

2.4. Insemination and pregnancy examination

During estrus, the mares were teased daily and the uterus and ovaries were examined by B-mode ultrasonography. The mares were artificially inseminated with 20 mL of raw semen from a fertile stallion when a follicle with a diameter greater than 40 mm accompanied by uterine edema was detected. The sperm concentrations ranged from 50,000 to 100,000 sperm/mm^3. Estrous mares were artificially inseminated every other day until ovulation.

The mares were examined for pregnancy on Day 12. Pregnant and nonpregnant mares were short cycled with $PGF_{2\alpha}$ and rebred with the goal of collecting data from two pregnancies per mare during the study.

2.5. B-mode and color Doppler ultrasonography

The Doppler measurements and calculations of uterine and ovarian blood flow were done as previously described [1]. Briefly, ultrasonographic examinations took place every 24 hours. An ultrasound machine (SSH-140A; Toshiba Co., Tokyo, Japan) equipped with a 7-MHz microconvex probe was used. The blood supplies of the left and right uterine