Intravaginal administration of sildenafil citrate increases blood flow in the bovine uterus

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Received: August 5, 2014       Accepted: January 9, 2015

Abstract

The aim of the study was to evaluate the influence of sildenafil citrate administrated intravaginally on the blood flow in the bovine uterus during dioestrus. Uterine blood flow was examined in six healthy adult cows. Sildenafil was administrated intravaginally to each cow between the 6th and 8th d of the ovarian cycle, in the form of vaginal suppositories containing 100 mg of active substance at a dose of 100, 200, or 300 mg per animal. Uterine perfusion was estimated by the colour Doppler examination, and obtained results were analysed with the Pixel Flux Software (Chameleon, Germany). Moreover, cardiovascular parameters were also evaluated. Animals were examined before and five times after drug application (two times at 15 min intervals, and three times at 2 h intervals). A placebo suppository was also given to the cows. The analysis of the intensity and velocity of blood flow in the uterus proved that sildenafil administrated intravaginally significantly increased blood flow in the uterus and the effect of increased perfusion was observed for 4 h and 30 min after administration. The effect of increased uterine perfusion was observed after low as well as high doses of sildenafil. Significant changes in the cardiovascular parameters were not detected. There were no changes in the uterine perfusion as well as in cardiovascular parameters after placebo administration.

Keywords: cows, blood flow, sildenafil citrate, colour Doppler.

Introduction

The influence of proper uterine perfusion on fertility has been previously evaluated in women (11, 16, 18, 19, 25, 28, 32-36). In some farm animals, the specificity of blood flow during different phases of the oestrous cycle and during pregnancy was successfully explored, and differences in perfusion during various phases of the ovarian cycle were noticed (3, 5, 6, 8, 17, 20-23, 26, 27, 29). Moreover, attempts to modify blood flow in the uterus have also been made both in humans and animals (7, 15, 29, 31, 32). Our previous study proved that the use of sildenafil citrate, administrated as an intrauterine infusion, increased perfusion of this organ in cows (15). Unfortunately, intrauterine route of sildenafil application carries serious limitations for practical and clinical use.

The evaluation of tissue perfusion with the colour Doppler technique allows to evaluate changes in blood flow not only in major arteries but also in the network of smaller vessels. It also makes it possible to detect more subtle changes in perfusion of particular parts of the organs (13, 14). Previously the lack of possibilities of objective evaluation of results obtained with the Doppler examination significantly reduced its usefulness for scientific research. Currently, however, the new Pixel Flux (PXFX) software designed for dynamic measurements of tissue and organ perfusion allows objective evaluation of results obtained with this technique, and for the past few years it has also been successfully applied in veterinary medicine (14).

On the basis of our previous results concerning the use of sildenafil in cows, also reports describing the usefulness of intravaginal administration of the drug as a suppository in animals, and the possibility for sensitive and objective evaluation of changes in tissue perfusion with the use of the modern software, the aim of this study was to evaluate the possibility of sildenafil...
Material and Methods

Animals. Six clinically healthy, adult, nonlactating Holstein-Friesian cows being in metoestrus, aged 3-5 years, and about 600 kg of body mass were used. For the synchronisation of the cycle, all cows were treated twice with 25 mg (i.m.) of natural prostaglandin, dinoprostone (Dinolyct, Pfizer) at intervals of 12 d. The reproductive status during the experiment was confirmed by analysis of the history (8 d after oestrus) and results of rectal examination (palpation and ultrasound examination) of the uterus and ovaries. In all cases, the presence of the mature corpus luteum on the ovary was confirmed. No pathological changes were found in any of the animals.

Doses, way of administration, and form of the drug administrated. Sildenafil citrate (Polpharma, Poland) was administered as an intravaginal suppository containing: 0.1 g of sildenafil citrate, 0.3 g of lactose, and cacao oil to 3.0 g. Suppositories were prepared by a professional pharmacist and stored at 4°C before application. All animals, in different trials performed during subsequent ovarian cycles (approximately 21 d interval), received one, two, or three suppositories, applied into the area of the outer mouth of the uterine cervix (cranial vagina). In all cases, catheterisation of the bladder was performed before drug application to reduce a risk of unwanted expulsion of active substance during urination. Additionally, a placebo trial was performed on each cow with a suppository with no active ingredient given in the same manner as the previously described.

Assessment of sildenafil citrate influence on the cardiovascular system. Assessment of sildenafil citrate influence on the cardiovascular system was performed using oscillometric measurements of the arterial blood pressure, electrocardiography (ECG), and echocardiography.

Measurement of systolic and diastolic pressure. Systolic and diastolic arterial pressures were measured with the PM 600 Vet (Mindray) oscillometric monitor. An indirect blood pressure was measured using a cuff placed at the basis of the tail (middle coccygeal artery). The cuff was repositioned as needed to acquire data acceptable for the internal sensor of the machine. All measurements were performed automatically. Blood pressure was monitored just before and every half an hour for 6 h after sildenafil citrate administration. Each measurement was repeated three times.

Evaluation of the ECG. ECG was performed using a base-apex lead with the Schiller AT-1 electrocardiograph (10). Electrocardiograms were recorded at speed of 25 mm/s and amplitude of 1 mV = 10 mm. The red electrode (negative) was attached to the left jugular furrow two-thirds of the way down the neck and the green electrode (positive) was placed just behind and to the left area of the apex. Heart rate (HR) and possible arrhythmias were monitored before and every half an hour for 6 h after sildenafil citrate administration.

Evaluation of the V max in the aorta. Before and six times after suppository administration, the blood flow in aorta was evaluated with the help of colour Doppler technique in the half an hour intervals. Simultaneously, the pulse was evaluated and echocardiography was performed. The maximum (V max) and mean (V mean) velocity in m/s in the aorta were measured during transthoracic echocardiography using pulse wave Doppler (PW) in the left ventricular outflow tract view from the left side. The probe was placed in the fourth intercostal space. Each measurement was repeated at least twice every half an hour.

Ultrasound examination of the uterus with the use of colour Doppler technique. For ultrasonographic examinations, the ultrasound system MyLab One (Esaote, Italy) equipped with 8 MHz rectal probe was used. During the examination, the probe was placed in the area of the uterine bifurcation and the colour signals imaging blood vessels were recorded. During the preliminary study, the importance of uterine perfusion was assessed by evaluating uterine blood flow during oestrus and comparing it to blood flow during metoestrus. An increase in uterine blood flow was observed in all animals during oestrus. This was in agreement with the reports of other researchers describing the differences in uterine perfusion during different phases of the ovarian cycle (1, 4, 6, 21). In all animals included in the experiment, the examination was repeated six times during one trial (before sildenafil administration, two times at intervals of 15 min, and three times at intervals of 2 h). Three trials were performed for all animals.

Evaluation of the results. For the evaluation of the results of the uterine blood flow, modern software Pixel Flux® (PFX, Chameleon, Germany) dedicated for dynamic tissue perfusion measurement was used. Blood flow was evaluated by measurements of the velocity and intensity in the defined region of interest (ROI). The mean flow intensity (I) inside a ROI as well as the mean flow velocity (V) was calculated according the procedures described by Dzięcioł et al. (14).

Statistical analysis. The deviation between values of velocity and intensity before and after sildenafil administration were analysed based on the Wilcoxon test. All data were displayed as the mean ± standard deviation and statistical significance was set at 5%. Normal distribution of data was confirmed by the K-S test (Kolmogorov-Smirnov test) (37).
Results

After intravaginal administration of the suppositories containing sildenafil citrate in all mentioned doses, an easily visible and confirmed by the computer analysis, significant increase in uterine blood flow was observed (Figs 1-6). There were no significant changes between perfusion parameters depending on the doses of sildenafil citrate applied. The duration of the effect, described as an increased uterine perfusion, was detected during all four measurements after sildenafil administration (for a duration of four and half hours), but in all animals, the last measurement (6 h after sildenafil administration) revealed a significant reduction of the evaluated parameters showing gradually reduced hyperaemia of the uterus. There were no significant changes noticed in the recorded cardio-vascular parameters in any of the experimental animals. The mean HR before (56 beats per min) and after sildenafil administration (58 beats per min) did not vary significantly. Additionally, there were no behavioural changes observed, including imbalance reported in a previous study after intrauterine administration (15). We did not observed significant changes in uterine perfusion, as well as in cardiovascular parameters after placebo administration.

Fig. 1. Blood flow velocity in bovine uterus before sildenafil administration (colours represents a direction of the blood flow; x axis presents number of video images recorded)

Fig. 2. Blood flow intensity in bovine uterus before sildenafil administration
Fig. 3. Blood flow velocity in bovine uterus after sildenafil administration (100 mg)

Fig. 4. Blood flow intensity in bovine uterus after sildenafil administration (100 mg)

Fig. 5. Uterine perfusion during metoestrus before sildenafil administration
Discussion

Sildenafil is an effective and selective inhibitor of phosphodiesterase type 5 (PDE5), which is responsible for degradation of cGMP (cyclic guanosine monophosphate) to guanosine monophosphate phosphodiesterase type 5. The mechanism of action of this drug leads to the relaxation of smooth muscle cells of blood vessels and in turn induces vasodilation, resulting in increased blood flow (9, 12). However, the main indication for the use of this medicine is the treatment of erection disorders in men; it was proven that the reproductive organs seem to be sensitive to sildenafil citrate both in males and females (2, 9). The positive effect of sildenafil on the uterine and placenta perfusion noticed in women, cows, and sheep also confirms this thesis (24, 31).

An increase in uterine blood flow confirmed good vaginal absorption of sildenafil. Temporary imbalance in cows that has been observed in the previous study after intrauterine infusion of sildenafil was probably connected with the effect of a sudden decrease in blood pressure, caused by sudden and significant dilatation of blood vessels (15). The lack of this kind of behaviour in the current experiment, as well as the lack of changes in cardiovascular parameters, with a concurrent easily observed increased perfusion of the uterus, could suggest a sufficient but slower absorption of sildenafil. This in turn can be connected with the weaker availability of the active substance gradually released from the suppositories. This effect should be considered as very positive and the proposed new model of sildenafil application should be a safer mode of administration for the animals.

Administration of sildenafil as an infusion into the uterine lumen could be contraindicated during every stage of pregnancy but increased perfusion of this organ could be desirable at this time and could carry potential benefits for the maintenance of pregnancy, which has been demonstrated and proven in women (33-36). Our study revealed that the intravaginal administration of the slowly released active ingredient from suppositories is equally effective, and also eliminates the limitations of intrauterine infusion.

Figs 5 and 6 present changes in the level of perfusion of the bovine uterus (increased number of pixels) after administration of 100 mg of sildenafil. Increased uterine perfusion is also demonstrated in the Figs 1-4 presenting chart changes in intensity and velocity of the blood flow after low dose of active substance. Similar changes were observed after higher doses (200 and 300 mg). The effectiveness of low doses of sildenafil could be beneficial in the context of possible further clinical applications of this kind of treatment.

Effectiveness of the new proposed route of vasodilator application and benefits of increased uterine perfusion for pregnancy maintenance, which has been described by many authors, carries the chance for the possible use of this drug as an additional, or in some cases even alternative method for uterine disorder treatment (28, 33-35). Taking into account the increasingly raising opinion on the need to reduce the use of antibiotics and hormones in food producing farm animals, future studies on the usefulness of sildenafil in uterine disorder treatment seem to be strongly indicated.
Conflict of Interest Statement: All authors declare that this article does not have any financial or non-financial conflict of interest.

Financial Disclosure Statement: This research was supported by statutory research and development activity funds assigned to the Faculty of Veterinary Medicine, Wrocław University of Environmental and Life Sciences.

Animal Rights Statement: The study was approved by the Local Ethics Committee.

Acknowledgements: Authors would like to thank Patrycja Barczyk for the suppositories preparation and to Polpharma S.A. for providing an active substance for the research procedures.

References


