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Canine uterine artery hemodynamic during Bromocriptine-induced estrus

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Abstract
Bromocriptine ((BRM); as a dopaminergic agent) reduces the serum prolactin, and is one of the most routine drugs to induce a successful estrus cycle in bitches. Some clinical side effects are accompanying this drug. This study aimed to investigate the hemodynamics of the uterine artery (UA) following administration of the increasing doses of BRM. In a case-control study, five non-pregnant bitches of mixed breeds in the anestrus stage received daily oral doses of BRM on days 1 and 2 (100, µg/kg), days 3 and 4 (200 µg/kg), and days 5 onward (400 µg/kg) until turning into proestrus. Three bitches, with expressed estrus without any intervention, were considered as control. The vaginal cytology, the ultrasound examination, and the serum progesterone (P4) assay were performed at 2-3 day intervals. Proestrus was induced within 6.6±1.17 days following BRM treatment. BRM significantly lowered the serum P4 to 15.1±0.78 compared to the control group (21.5±1.13 ng/mL) during induced estrus. BRM significantly changed UA hemodynamics over the days before proestrus. Mean UA pulse index, resistance index, and peak systolic velocity in BRM-induced estrus were significantly lower than the control group. The results of this study showed lower serum P4 levels and some alterations in the canine uterine hemodynamic during BRM-induced estrus compared to naturally expressed estrus. Induced cycle in dogs with lower serum P4 levels and altered UA hemodynamics must be considered for subsequent pregnancy outcomes in the BRM induced-estrus.

Keywords: Dogs, Bromocriptine; Estrus Cycle; Uterine Artery

Introduction
The bitches experience a long-term (3-10 months) anestrus in which their reproductive functions are minimized. This period varies among different dogs, and even among heat cycles of the same bitch (Verstegen et al., 1999). Owners of elite breeds usually ask veterinarians to shorten inter-estrus intervals. Various medications have been employed to do this among which dopamine agonists, including Bromocriptine (BRM), inhibit prolactin (PRL) secretion by directly stimulating dopamine receptors (Jöchle et al., 1989; Kutzler, 2007; Kutzler, 2018). PRL has been shown to have various cardiovascular effects including vasoconstriction and positive chronotropic impact in laboratory animals (Manku et al., 1973). It has also led to coronary, mesenteric, renal, and iliac artery constriction when infused intravenously (Molinari et al., 2007). A positive relationship has also been

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established between PRL levels and essential hypertension, and oral administration of BRM has significantly decreased the blood pressure of those patients by suppressing plasma PRL (Stumpe et al., 1977).

The reproductive organs are very dynamic either during normal or pathological conditions. Due to ovarian function, hormone profile alterations may change the vascular hemodynamics in the uterus and ovaries during different stages of the estrus cycle; the ovarian blood perfusion increases over ovulation in bitches (Bergeron et al., 2013). The severe hemodynamic changes are occurring within the uterine artery (UA) during pregnancy (Blanco et al., 2011). Administration of cabergoline in hyperprolactinemic patients significantly decreased uterine, spiral, and intraovarian artery PI and RI (Temizkan et al., 2015). The color doppler technique enables evaluation of the uterine vascular hemodynamics in terms of peak systolic velocity (PSV), end-diastolic velocity (EDV), pulse index (PI), and resistance index (RI) which may help to have a clear imagination about perfusion status following administration of BRM.

Accordingly, we hypothesized that estrus manipulation using BRM may alter uterine perfusion and change the UA hemodynamic indices due to its suppressive impact on PRL and probably other hormonal changes. To our knowledge, the role of BRM and its subsequent induced estrus on the canine uterine vascular parameters have not been evaluated. Therefore, the present study aimed to investigate the canine UA hemodynamic during the BRM-induced estrus.

Materials and Methods

Animals

The study was carried out at the veterinary clinic of Shahrekord University. Eight crossbreeds, healthy dogs (weight: 18-23 kg; age: 3- 5 years) were used in this study after being evaluated physically and recording their signalments and reproduction histories. The animals received a dose of rabies vaccine (Biocan R, Biovita, Czech Republic) and a single dose of Ivermectin (Ivectin 1%, Razak, Iran). Vaginal smears were evaluated to confirm that the bitches are in the anestrus stage. All the procedures were approved by the ethics committee for research on animals at Shahrekord University.

Doppler Ultrasonography

The dogs were off-fed eight hours before examinations and just received water *ad libitum*. Using a B-mode ultrasound examination, the normal uterus was located, followed by a color doppler evaluation (7.5 MHz, Mindray.z6.vet, China). The UA was located and the appearance of the streaming wave was recorded using pulse waves and calculated the respected vascular parameters (Figure 1) including PSV (cm/s), EDV (cm/s), RI= [(PSV − EDV)/PSV], and PI (Alvarez-Clau & Liste, 2005).

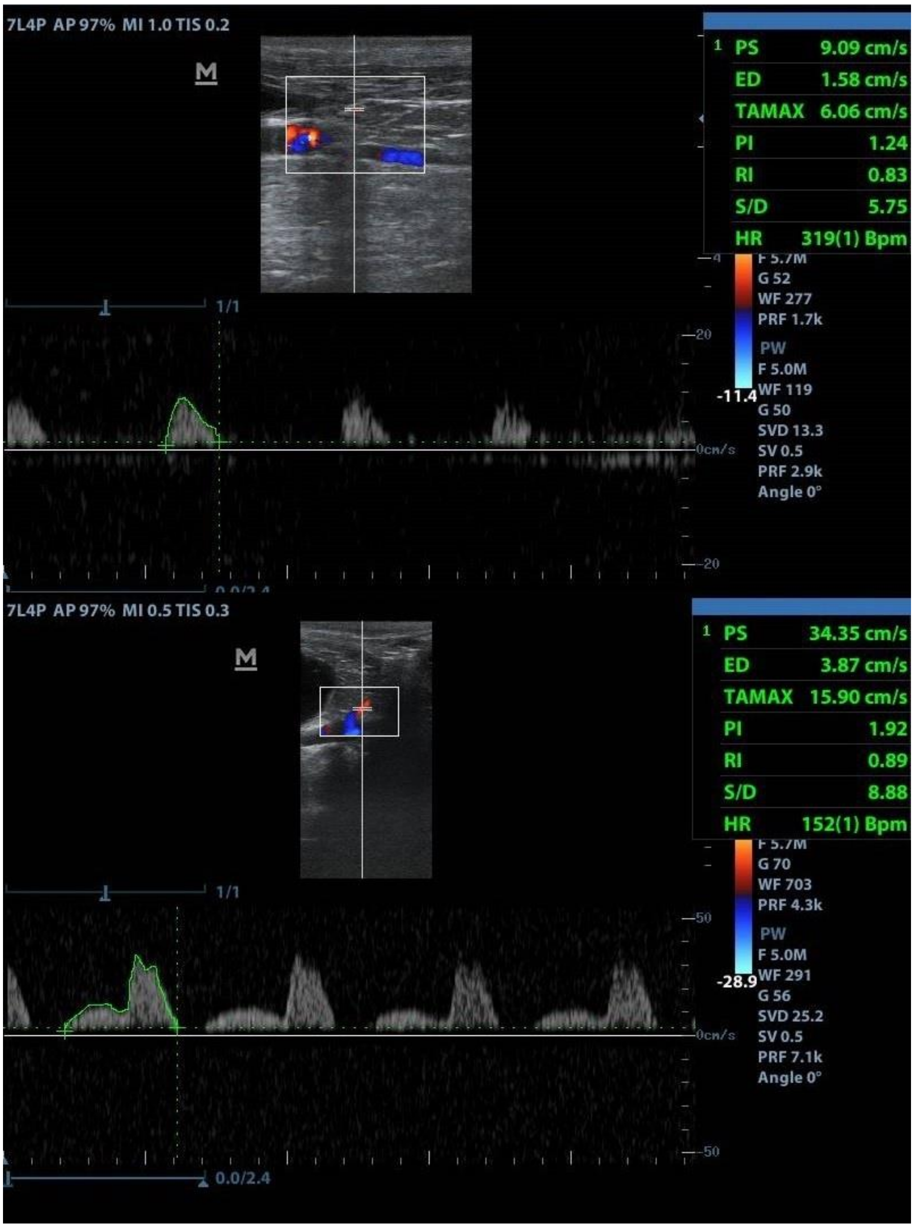

Fig 1. The color Doppler images of mid uterine artery from two different dogs before treatment (above) and at the diestrus (below) phase during the experiment.

Evaluation of vaginal smear

At the time of each examination, vaginal smears were collected, stained with Giemsa, and evaluated using a light microscope. Proestrus and diestrus stages were determined using vaginal smears. Figure 2 shows the red blood cells with intermediate superficial cell in proestrus, anuclear and large superficial cells in estrus, and neutrophil accumulation with anucleated and intermediate superficial cells in diestrus. Anestrus phase was recognized with parabasal cells in the smears (Johnston et al, 2001).

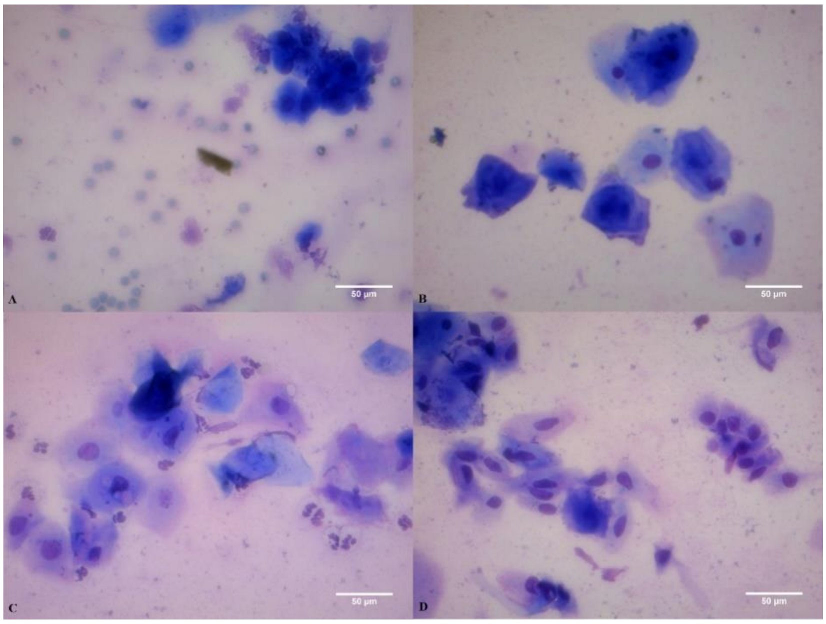

Fig 2. Photographs of canine vaginal smears taken at different phases of estrus cycle (A: proestrus; B: estrus; C: diestrus; D: anestrus).

Serum P4 assays

Serum samples were collected at the times of examination and stored at -70°C until assay. The P4 concentrations were measured using ELISA (AccuBind ELISA, Monobind, Inc., LakeForest, CA, USA). Inter and Intra assays coefficients of variation for 20 samples were 7.5 and 3.8 %, respectively. The LH surge and the ovulation time were estimated based on the guideline described previously (Johnston & Root, 1995).

Experimental design

The experiment was designed as a case-control study with repeated measurements of the respected factors on defined days of the experiment. Three bitches were assigned as control and monitored to express their estrus naturally without any intervention. The five remaining bitches were assigned as treatment and received increasing daily oral doses of BRM (2.5 mg tablet, Iran Hormone®); days 1 and 2 (100, µg/kg), 3 and 4 (200 µg/kg), and days 5 onward to proestrus expression time (400 µg/kg). The vascular changes in the treatment group before proestrus expression were compared with before treatment. The vascular parameters during the period of estrus expression were compared between treatment (BRM-induced estrus) and control (naturally expressed estrus) groups in a repeated measure design. The dogs were sampled for vaginal cytology, ultrasound examination, and serum P4 assay every 2-3 day intervals.

Data analysis

Before proestrus expression, data after treatment commencement were compared to samples before treatment in the treated group with one-way ANOVA and the Dunnet multiple comparison test. The data obtained during cycles were compared between treatment and control groups using the mixed model analysis to find the effects of treatment and day of sampling on the different variables. The *pdiff* post hoc test compared the least square means and standard error of means (LSmeans±SEM). The Pearson correlation test was applied to explore any correlation between the vascular indices and the serum P4 levels. The p values less than 0.05 were considered significant. Statistical Analysis System (SAS 9.2.4) was used for analysis (SAS Institute Inc, 2009).

Results

All 5 bitches were in proestrus following administration of increasing doses of BRM (6.6±1.7 days). Table 1 shows the effects of BRM treatment on UA before proestrus occurrence. The indices of PI, RI, EDV, and PSV were significantly under influence of BRM treatment before proestrus expression. The highest mean PI, RI, PSV, and EDV were recorded at 3-5, 3, 10, and 7 days after treatment, respectively.

The BRM-induced estrus was associated with lower levels (ng/mL) of the serum P4 compared to normally expressed estrus (15.1±0.78 vs 21.5±1.13). Figure 3 shows lower serum levels of P4 in the treatment compared to control groups through the cycle, with significant differences from days 12 to 16 (P<0.05).

Table 1. The different vascular parameters of the canine uterine artery during the administration of ascending oral doses of Bromocriptine†.

	Before treatment	Days after treatment		
		3	5	7
PI	1.07±0.04	1.2±0.4*	1.2±0.06	0.85±0.09*
RI	0.78±0.01	0.85±0.02*	0.83±0.02	0.64±0.03*
PSV (cm/sec)	12.2±0.19	13.6±0.21*	14.1±0.29*	15.8±0.43*
EDV (cm/sec)	2.7±0.2	2.1±0.23*	2.5±0.32	5.3±0.47*

Star mark (*) indicates significant differences in parameters compared to before treatment (P<0.05). PI: pulse index, RI: resistance index; PSV: peak systolic velocity, EDV: end-diastolic velocity. †least square means ± SEM.

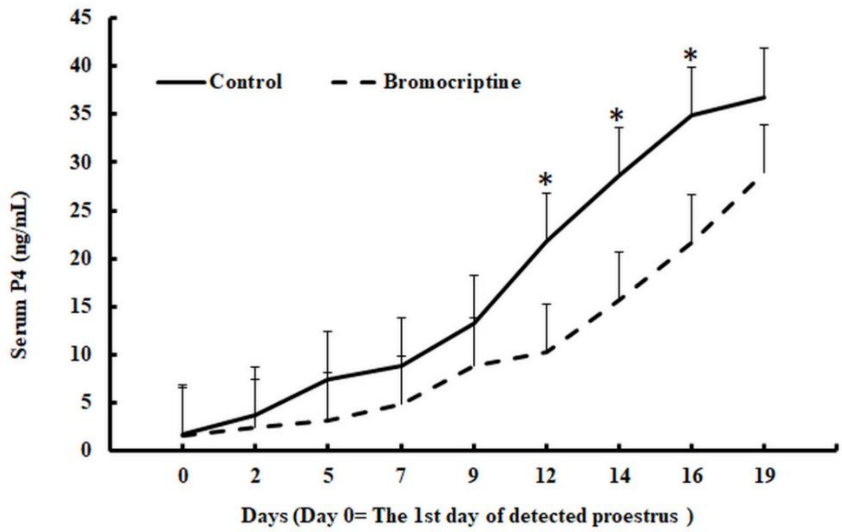

Fig 3. The serum progesterone concentrations (LSmean±SEM) during the canine estrus cycle, either naturally (n=3) or induced by ascending doses of Bromocriptine administration (n=5). *indicates a significant difference at each time point between the two groups (P<0.05).

The mean PI (1.24±0.01 vs 1.3±0.02; P<0.0001), RI (0.83±0.004 vs 0.85±0.005; P=0.0007), and PSV (22.2±0.18 vs 25.4±0.23; P<0.0001) were lower in BRM induced-estrus compared to naturally expressed estrus. The mean EDV was not significantly different in treatment and control (3.6±0.07 vs 3.7±0.09; P=0.2156). Figure 4 shows increasing levels of PI throughout the estrus cycle; the higher levels of PI from day 16 after proestrus were recorded in the control group compared to the treatment group. A significantly higher value of RI was seen on day 14 in control than in treatment. An increasing trend of PSV was recorded in the control group that was higher than the treatment group at different time points. The EDV index was higher in control compared to treatment on days 9 and 12 after proestrus expression.

The Pearson correlation test shows a significant and positive correlation between serum P4 levels and different uterine vascular indices; PI: r=0.5, p<0.0001; RI: r=0.255, p=0.0346; PSV: r=0.85, P<0.0001 and EDV: r=0.29, P=0.0147.

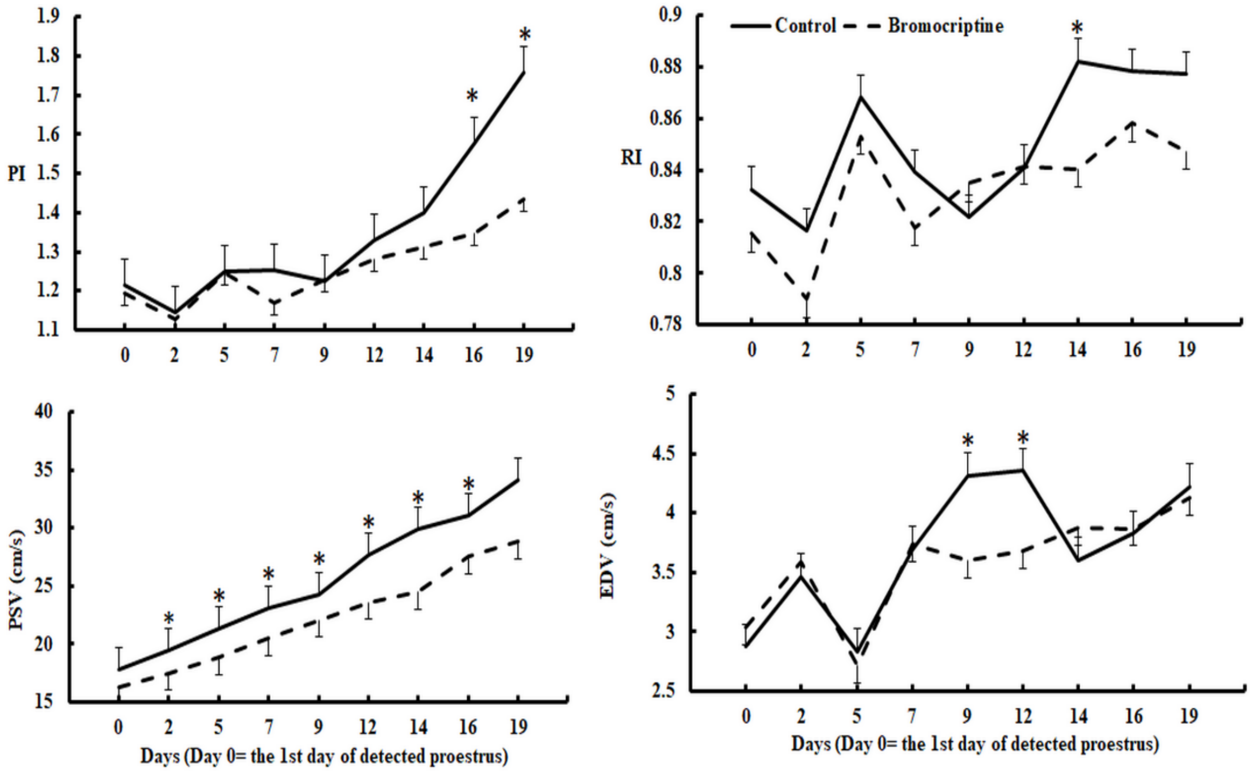

Fig 4. The hemodynamic indices of canine uterine artery (LSmean±SEM) during estrus cycle, either naturally (n=3) or induced by ascending doses of Bromocriptine administration (n=5). *indicates a significant difference at each time point between the two groups (P<0.05).

Discussion

The ascending doses of BRM effectively induced the proestrus in the bitches. Different protocols are used for canine estrus induction; including gonadotropins, prostaglandin F2α, and anti-prolactin agents (Cabergoline, BRM) (Kutzler, 2005). The most problem in the common protocols for estrus induction is the large variation in the effective time and number of injections or administrations. On the other hand, the side effects of some drugs include nausea and vomiting may reduce the interest of owners in using the drugs. Previous studies have shown that BRM reduces the plasma levels of PRL and P4 by daily administration of 5 to 250 µg/kg in either pseudopregnant (Gobello et al., 2001) or anestrus (Okkens et al., 1985) beagle bitches. Okkens et al. (1985) used daily 250 µg/kg administration of BRM and caused a 35 % decrease in the duration of the anestrus stage and a 78 % reduction in the luteal stage of the bitches. In the study of Beijerink et al. (2003), daily oral administration of 20 and 50 µg/kg BRM efficiently reduced plasma PRL concentrations compared to 5 µg/kg. However, all three doses efficiently shorten the inter-estrus interval in the beagle bitches. Kooistra et al. (1999) reported an increase in FSH pulses following BRM treatment and describe it as a factor for shortening inter-estrus interval. They reported no effect of BRM on LH pulses or amplitude that indicates non-luteotropic effects of BRM. The current study showed the effectiveness of ascending doses of BRM on the estrus expression while reducing the serum P4 levels during induced estrus.

In the present study, during BRM treatment significant changes were observed in the vascular indices, especially while animals turned to proestrus. The decreasing trends of RI and PI and increasing state of PSV and EDV were recorded during BRM treatment until proestrus appearance. This effect can be related to the direct effect of BRM, as a dopaminergic agent, with interactions of α2-adrenoreceptors (Villalón et al., 2003) or can be due to indirect effects of BRM by alterations in steroid profile (Resnik et al., 1977) that was confirmed in the present study. Bollwein et al. (2016) confirmed the influence of estrogen and progestogen on uterine vascular perfusion.

The UA indices in the control group of our study confirm the results of the Nogueira et al. (2017) who showed similar changes throughout the estrus stages in the Beagle dogs. The difference between net values of PSV and EDV in our study with Nogueira et al. (2017) may relate to the breed of dog or their demographic properties. BRM-induced estrus was associated with changes within uterine vascular indices compared to the normal cycle in the present study. However, the changes within each group were significant on different days. As the mean serum P4 was significantly lower in the BRM-induced cycle, in the present study, the reduced PI and RI can be due to alteration in the P4 profile. This finding is in line with the study of Bollwein et al. (2004) who showed higher PI in altrenogest (a progestogen supplement) induced estrus in mares. Previously, Weiner et al. (1993) reported that induced cycles in humans are associated with decreased impedance in uterine blood circulation. Our results are in line with the study of Nogueira et al. (2017), which showed changes in the UA indices during different stages of the normal estrus cycle in Golden Retriever bitch that all indices were increased from proestrus to anestrus. Batista et al. (2013) showed that during the puerperium, the RI increases as the bitch is going to anestrus status. The PSV values were lower in BRM-induced estrus than in the animals with natural estrus throughout the experiment. EDV was significantly higher in the natural cycle compared to BRM-induced estrus during possible days of cytological diestrus appearance. Blanco et al. (2009) have shown that induction of abortion in beagle bitch cause to increase in RI and P/D ratio compared to normal parturition, which has been described by progesterone deprivation (Blanco et al., 2008). Bollwein et al. (2000) showed the highest values of RI on the day of ovulation and the lowest values 3 to 1 day before estrus expression in the bovine UA which is in line with our study. However, our results that showed a correlation between vascular indices and the serum P4 are in contrast with Bollwein et al. (2000) study that reported no correlation between RI and P4 concentrations in cows.

Increased PSV with constant PI in UA is associated with ovulation in human (Campbell et al., 1993) and high values of uterine PI was associated with women's infertility (Tinkanen et al., 1994). With alteration in the serum P4 and the vascular indices in BRM-induced estrus in the current study, subsequent pregnancy outcomes in the treated bitched should be considered.

BRM administration alters vascular properties in bitch while she enters in proestrus. The results of the present study have shown lower serum levels of P4 during induced estrus with ascending doses of BRM. The BRM-induced estrus in bitch is associated with alteration in PI, RI, and PSV throughout the cycle compared to the normal cycle.

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Conflict of interest

The authors declare no conflict of interest.

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همودینامیک شریان رحمی سگ در طول فحلی القاء شده به وسیله‌ی بروموکریپتین

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چکیده

بروموکریپتین ((BRM)؛ به عنوان یک عامل دوپامینرژیک) پرولاکتین سرم را کاهش می‌دهد و یکی از معمول‌ترین داروها برای القای یک چرخه‌ی فحلی موفق در سگ‌ها است که برخی از عوارض جانبی بالینی مرتبط با این دارو است. این مطالعه با هدف بررسی همودینامیک شریان رحمی (UA) پس از تجویز دوزهای افزایشی BRM انجام شد. در این مطالعه پنج سگ غیر آبستن از نژادهای مخلوط در مرحله‌ی آنستروس دوزهای خوراکی BRM را در روزهای ۱ و ۲ (۱۰۰ میکروگرم بر کیلوگرم)، روزهای ۳ و ۴ (۲۰۰ میکروگرم بر کیلوگرم) دریافت کردند. روز ۵ به بعد (۴۰۰ میکروگرم بر کیلوگرم) تا بروز پرواستروس. سه سگ با فحلی بدون مداخله به عنوان شاهد در نظر گرفته شدند. سیتولوژی واژینال، معاینه اولتراسوند و سنجش پروژسترون سرم (P4) در فواصل ۲-۳ روز انجام شد. پروستروس در ۶/۱۷±۶/۱ روز پس از درمان BRM القا شد. BRM به طور قابل توجهی P4 سرم را به ۱۵٫۱±۰٫۷۸ در مقایسه با گروه کنترل (۱/۱۳ ± ۲۱/۵ نانوگرم در میلی‌لیتر) در طول فحلی القایی کاهش داد. BRM به طور قابل توجهی همودینامیک UA را در طی روزهای قبل از پروستروس تغییر داد. میانگین شاخص پالس UA، شاخص مقاومت و حداکثر سرعت سیستولیک در فحلی ناشی از BRM به طور قابل توجهی کمتر از گروه کنترل بود. نتایج این مطالعه سطوح پایین‌تر P4 سرم و برخی تغییرات در همودینامیک رحم سگ در طول فحلی ناشی از BRM در مقایسه با فحلی با بیان طبیعی نشان داد. چرخه‌ی القایی در سگ‌هایی با سطوح سرمی P4 پایین‌تر و همودینامیک UA تغییر یافته، می‌بایست برای پیامدهای بارداری بعدی در فحلی ناشی از BRM در نظر گرفته شود.

کلمات کلیدی: سگ، بروموکریپتین، چرخه‌ی فحلی، شریان رحمی

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