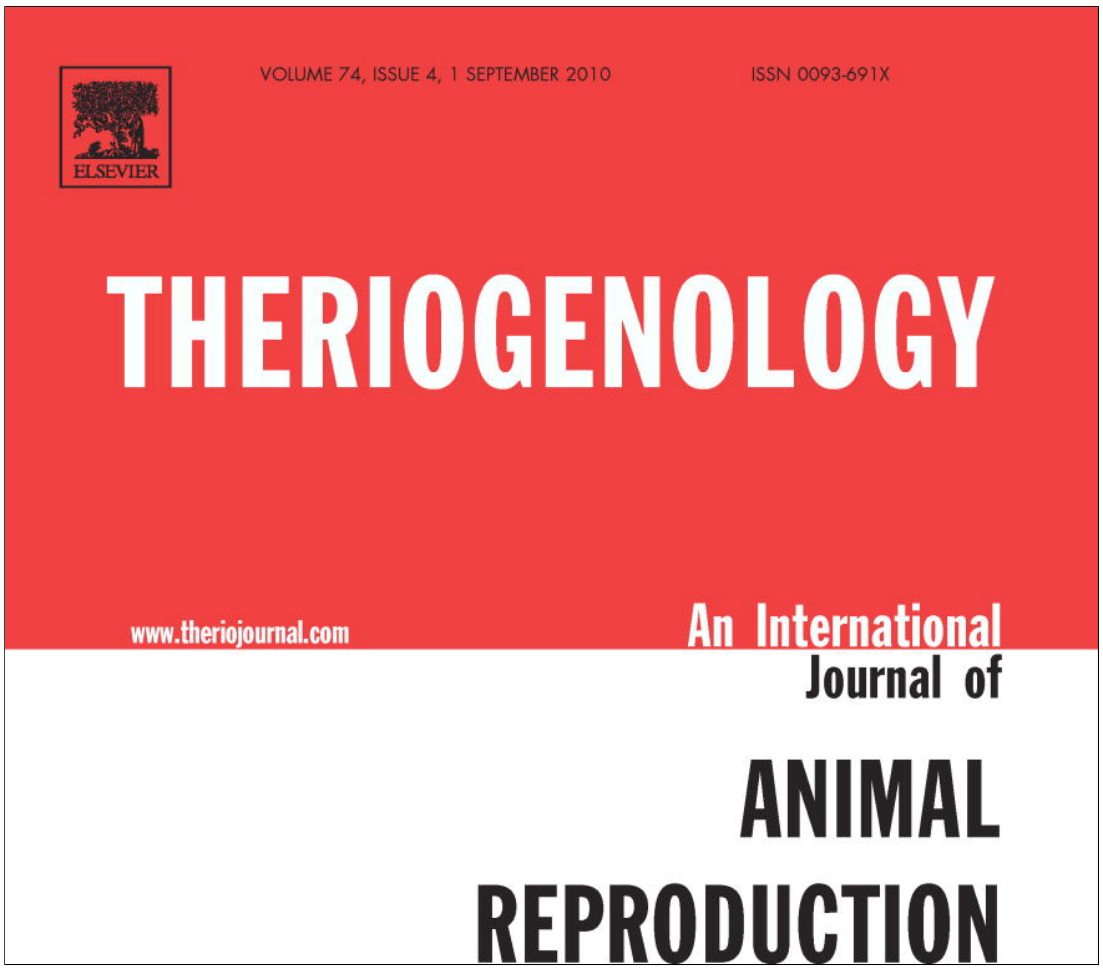

Available online at www.sciencedirect.com

ScienceDirect

Theriogenology 74 (2010) 672–681

Theriogenology

www.theriojournal.com

Aglepristone (RU534) administration to non-pregnant bitches in the mid-luteal phase induces early luteal regression

A. Polisca[a], L. Scotti[a], R. Orlandi[a], G. Brecchia[b], M. Maranesi[b], M. Zerani[c], C. Boiti[b,*]

[a] *Dipartimento di Patologia, Clinica e Diagnostica Veterinaria, Sezione di Ostetricia e Ginecologia, Università di Perugia, via S. Costanzo 4, Perugia I-06123, Italy*

[b] *Dipartimento di Scienze biopatologiche ed Igiene delle produzioni animali e alimentari, Sezione di Fisiologia Veterinaria, Laboratorio di Biotecnologie fisiologiche, Università di Perugia, via S. Costanzo 4, I-06123, Italy*

[c] *Scuola di Scienze mediche veterinarie, Università di Camerino, via Circonvallazione 93, Matelica (MC) I-62032, Italy*

Received 31 August 2009; received in revised form 25 March 2010; accepted 25 March 2010

Abstract

The effect of the antiprogestagen aglepristone (10 mg/kg bw), administered at days 29 and 30 following the estimated day of LH surge (day 0), on corpora lutea (CL) function was examined during the diestrus phase of non-pregnant bitches. Aglepristone shortened ($P < 0.01$) the luteal phase and complete luteolysis (progesterone <2 ng/mL) was observed at days 40.8 ± 3.5 and 71.5 ± 4.6 (means ± SD; n = 9/group) in treated and control bitches, respectively. Peripheral estradiol-17β concentrations declined from 91.5 ± 14.3 pg/mL at day 9 to 50 pg/mL at day 18, remaining at approximately the same levels thereafter in both treated and control bitches. Intraluteal *in vitro* synthesis of progesterone and estradiol-17β released by CL explanted at day 38 from control bitches (511.9 ± 285.6 and 40.7 ± 17.2 pg/mg protein, respectively) did not differ from that of treated. From day 38, intraovarian hemodynamic variables (arterial blood flow, systolic peak, and end-diastolic velocities), monitored by color-coded and pulsed Doppler, decreased more steeply ($P < 0.01$) in aglepristone-treated (n = 4) than in control (n = 4) bitches, whereas the resistance index increased ($P < 0.01$) in treated animals. All the blood flow parameters were undetectable at 60 ± 3.6 and 68 ± 2.0 days (medians ± SD) after LH peak in treated and control bitches, respectively. In conclusion, aglepristone administration to dogs during the mid-luteal phase markedly accelerates the luteolytic process which is accompanied by a parallel decline in ovarian blood flow supply with a shift from approximately 8 to 10 days.

Keywords: Progesterone; Estradiol-17β; Ultrasonography; Diestrus

1. Introduction

The corpora lutea (CL) are transient endocrine glands that secrete progesterone, a steroid hormone which is necessary for implantation and maintenance of pregnancy in all mammalian species [1–3]. However, whenever pregnancy fails to occur, the CL undergo luteolysis, a process that greatly reduces the length of diestrus in most species. In contrast, the length of the progesterone secreting luteal phase is almost similar in pregnant and non-pregnant canids, a characteristic that differentiates this species from other mammals [4–7]. In addition, during the first half of diestrus, up to approximately 30 days after ovulation, the CL function is independent from the luteotrophic support provided

* Corresponding author. Tel.: +390755857654; fax: +390755857654.
E-mail address: cristiano.boiti@unipg.it (C. Boiti).

0093-691X/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.theriogenology.2010.03.021

by the pituitary, which subsequently becomes indispensable [8–12].

Little is known about the endocrine-regulating mechanisms in the bitch that program the function of CL, activating their luteotrophic dependency on the pituitary at mid-diestrus, and triggering luteal regression at late-diestrus [13]. Whereas the number of CL receptors for LH, prolactin, and estradiol-17β do not change throughout diestrus, the density of progesterone receptor (PR) increases during the early- and late-luteal phase [12,14], suggesting an endocrine-mediated modulation of its expression. Both progesterone and estradiol-17β act at hypothalamus-pituitary level to regulate gonadotropin and prolactin secretion [15–17] as well as several aspects of ovarian physiology, including those related to follicular growth and granulosa cell function [18]. In both pregnant and non-pregnant dogs, during the diestrus phase, peripheral plasma estradiol-17β concentrations remain elevated [19–21]. Circulating estrogens likely derive from the ovary given that aromatase positive cells were found only in luteal cells [22], but the question whether estradiol-17β does exert any paracrine role in maintaining CL function as reported for other species, such as the rat and the rabbit, remains to be proven.

Both mifepristone (RU486) and aglepristone (RU534) bind with high affinity to nuclear PR at peripheral and central levels without any progestin-like activity [23–25]. Thus, by competing with endogenous cognate receptors, these progesterone antagonists can be used to functionally inhibit progesterone actions, including embryo implantation [26–29]. In bitches, both progesterone antagonists can prevent unwanted gestation when administered before embryo implantation and induce resorption, abortion, or parturition [30–36]. However, treatment with RU486 at different intervals after proestrus bleeding did not affect luteal function of bitches with overt symptoms or previous history of pseudopregnancy [37]. In addition, mifrepristone given at 2-day intervals for 80 days soon after proestrus bleeding did not modify the luteal function of a hysterectomized dog [37]. Moreover, aglepristone administered to non-pregnant bitches in the early-luteal phase, within 12 days after ovulation, affected progesterone secretion but not sufficiently to shorten the length of the luteal phase [38]. Thus, increasing evidence suggests that the action of these antiprogestagen compounds on CL function depends not only by treatment protocols but also by the specific reproductive phase at which they are administered and the presence of the uterus. However, it is still unclear whether progesterone antagonist exercises any action on luteal function of healthy bitches at later stages of diestrus, and its precise mechanisms of action, direct or indirect, have yet to be defined.

The color Doppler ultrasonographic technique is a useful, non-invasive tool routinely used in small animal clinics that provides real time information on critical hemodynamic parameters which help in monitoring the functional status of the reproductive organs and several physio-pathological conditions from the earliest stage of pregnancy [39]. Therefore, the aims of the present work were to evaluate the effect of aglepristone administered in the mid-luteal phase of non-pregnant dogs on CL and ovarian function as assessed by progesterone and estradiol-17β peripheral blood concentrations and *in vitro* synthesis together with real-time hemodynamic changes of the ovary.

2. Materials and methods

2.1. Animals

All the 22 bitches included in this study, which spanned over a period of two years, were registered in the day-hospital service provided by the Obstetric and Gynecology unit of the Veterinary Teaching Hospital annexed to the Faculty of Veterinary Medicine, with the informed consent of their owners. The bitches, twelve German shepherds aged 4–6 years and ten mixed breeds aged 2–5 years and weighing between 12 and 25 kg, were judged healthy on the basis of routine clinical examinations. According to the clinical history of each dog, the inter-estrus intervals of previous cycles were normal. From the first appearance of vulvar serosanguineous discharges, indicating the onset of proestrus, vaginal smears were taken daily from each bitch and promptly examined upon hematoxylin-eosin staining until the first day of cytological diestrus. The LH peak (day 0) was estimated as 8 days prior to the onset of the characteristic metestrus cells (foam, parabasal, and small intermediate) [40]. The protocols involving the care and use of the animals for these experiments were approved by the Bioethic Committee of the University of Perugia.

2.2. Treatment protocol

Bitches were randomly assigned to either control or treated groups. At both days 29 and 30 after LH peak, control bitches (n = 9) received saline solution (0.3 mL/kg bw, subcutaneously), whereas treated ones (n = 9) received aglepristone (Alizine®, Virbac Laborato-

ries, Carros, France) at the dose of 10 mg/kg bw for two subsequent days, subcutaneously.

2.3. Blood sample collection

Blood samples (~2 mL) were collected from each dog three times a week from day 9 to day 73 after the estimated LH peak by venipuncture of the cephalic vein. Blood samples were obtained before saline or aglepristone treatment and before ultrasonographic scans. Upon collection, blood samples, drawn into tubes without anticoagulant, were centrifuged (3000 × g for 15 min) and sera stored at −20 °C until assayed for progesterone and estradiol-17β.

2.4. Tissue collection, processing, and in vitro incubations

At day 38 after the estimated LH, four additional German shepherd bitches, 2 controls and 2 treated with aglepristone as above specified, underwent elective ovariohysterectomy under general anesthesia induced by propofol (4 mg/kg bw) and maintained with isoflurane. Upon removal, the reproductive tracts were thoroughly washed with saline and the CL promptly excised from ovaries and, after careful dissection of non-luteal tissue by fine forceps, immediately processed for *in vitro* studies. The CL (treated: n = 12; controls: n = 12) explanted from both ovaries of each bitch were placed into Petri dishes containing culture medium 199 GIBCO (Grand Island, NY, USA) with Earles Balanced Salt Solution (GIBCO), 2.2 mg/mL sodium bicarbonate, 2.3 mg HEPES (Sigma (St Louis, MO, USA), and 3% BSA (Sigma), referred to here as M199 and chopped into small pieces using fine forceps. The tissue samples of each CL were randomly distributed (100–110 mg/well) into incubation wells (Becton Dickinson Co., Clifton, NJ, USA) filled with 1 mL of M199. The culture plates were incubated at 37 °C in air with 5% CO_2 as reported elsewhere [41]. Media and CL of each well were collected after 4 h of incubation and stored immediately at −80 °C for later determination of progesterone and estradiol-17β levels.

2.5. Hormone assays

Progesterone and estradiol-17β concentrations were assayed by RIA as previously reported [42]. The tritiated hormones were purchased from Amersham Biosciences (Little Chalfont, Bucks, UK), while the corresponding antisera came from Sigma. Briefly, 1 ml of serum samples and 1 ml of culture media were extracted with 5 mL diethyl ether on a vortex mixer for 5 min and the organic fractions transferred into glass tubes and evaporated to dryness under nitrogen. The extracts were resuspended in 1 mL of assay buffer (Na_2HPO_4 74.5 mM, EDTA-Na_2 12.5 mM, gelatine 0.1%, NaN_3, pH 7.4). In a typical assay, a tube containing 100 μL of extract, 100 μL of tritiated hormone, 100 μL of specific antiserum, and 200 μl of assay buffer was incubated overnight at 4 °C. Antibody-bound- and free-hormone were separated with 500 μl of dextran-coated charcoal mixture (Dextran 70, 0.0625% and Norit A, 0.625%) in assay buffer. After incubation (4 °C for 10 min) and centrifugation (1 500 × g for 10 min), the supernatant, containing the antibody-bound-hormone, was transferred into a vial, containing 3 mL of scintillation liquid, for the radioactivity determination in a β-counter. All determinations were done in duplicate. Intra- and inter-assay coefficients of variation, and minimum detectable doses were progesterone: 6.9%, 12.9%, and 0.12 ng/mL; estradiol-17β: 9,1%, 11,3%, and 15 pg/mL. The recovery rates for progesterone and estradiol-17β were 91.4 ± 3.5%, 89.4 ± 5.7%, respectively.

2.6. Ovarian blood flow by ultrasonography

The intra-ovarian blood flow was monitored three times a week in four control and four treated German shepherds, from day 9 following LH peak to day 73, by color-coded and pulsed Doppler ultrasonography as previously described [43], using a Sonoace 8800 (Full Digital Medison Inc., Austria) equipped with a 6.5 MHz-micro-convex probe. The ultrasound scans, performed by the same operator to minimize variability, were always carried out at the same time (9:00 to 12:00 AM) and lasted about 40 min for each bitch. Color-coded Doppler ultrasonography was used to visualize and estimate intra-ovarian vascularization. Thereafter, a pulsed wave (PW) Doppler sample volume was placed in the centre of the color-coded blood flow and the waveforms of at least three consecutive cardiac cycles were recorded to calculate by in-built-in software the systolic peak velocity (SPV), the end-diastolic velocities (EDV), as well as pulsatility index (PI) and resistance index (RI). The Power Doppler (PD) was run with a repeat frequency of 2.50 kHz and color gain between 65 and 85. The angle of insonation between the Doppler stream and the direction of the vascular segment was manually aligned and the measure of blood flow velocity was automatically adjusted. Measurements with an angle >60° and <20° were disregarded. For each ovary, three values for the PW and three images for the PD were acquired, as previously described, and used for quantitative analysis.

2.7. Statistical analysis

Data relative to *in vivo* treatment effects on hormone levels were analyzed by one-way ANOVA for repeated measures with time and treatments as variables followed by Student-Newman-Keuls test. $P < 0.05$ was considered statistically significant. For the purpose of this work, partial luteolysis was defined as a 50% decrease in serum progesterone concentrations from the mean values observed before treatment, between days 9 and 29 after LH peak, while complete luteolysis was defined as the failure of CL to secrete progesterone with consequent drop in blood levels to below 2.0 ng/mL, a level found in anestrus bitches [14]. Data relative to *in vitro* steroid hormone production were analyzed by Student's *t* test.

For the analysis of correlation between luteolysis and ovarian blood flow, as assessed by blood progesterone concentrations and power Doppler data, respectively, post-treatment values were expressed as relative percentages of the corresponding pre-treatment values that were averaged up to day 29 after the LH peak and set to 100. These data and the other hemodynamic parameters were analyzed by a non-parametric Kruskal Wallis test followed by Student-Newman-Keuls test. $P < 0.05$ was considered statistically significant.

3. Results

The administration of aglepristone did not cause any apparent side effects in the treated bitches throughout the experiment. During the next six months of follow up, no significant clinical signs were reported by the owners or observed in routine physical examinations.

3.1. In vivo studies

Before treatment, the mean serum progesterone concentrations averaged 31.3 ± 2.6 and 30.5 ± 3.4 ng/mL in control (n = 9) and treated group (n = 9), respectively (Fig. 1A). The length of the luteal phase of treated bitches was shorter ($P < 0.01$) than that of control bitches (Fig. 1A). Luteolysis began at day 32.8 ± 2.6 (n = 9) after LH peak in treated bitches and at day 41.9 ± 3.5 (n = 9) in controls. Complete luteolysis was observed at day 40.8 ± 3.5 (n = 9) in treated bitches and 31 days later ($P < 0.01$) in controls, at day 71.5 ± 4.6 (n = 9) (Fig. 1A).

The mean serum estradiol-17β concentrations were relatively high at day 9 after LH peak (91.5 ± 14.3 pg/mL, n = 18), but gradually declined to 45 to 50 pg/mL at day 18, remaining at approximately the same levels thereafter (Fig. 1B). Serum estradiol-17β levels did not differ in treated and control bitches before and after aglepristone administration, during the time frame examined up to day 73 (Fig. 1B).

3.2. In vitro CL function

Intraluteal basal *in vitro* synthesis of progesterone and estradiol-17β released into the culture medium by CL explanted from bitches treated with aglepristone (n = 12; progesterone: mean ± SD = 437.2 ± 228.8 pg/mg protein; estradiol-17β: mean ± SD = 51.7 ± 22.3 pg/mg protein) did not differ from that of controls (n = 12; progesterone: mean ± SD = 511.9 ± 285.6 pg/mg protein; estradiol-17β: mean ± SD = 40.7 ± 17.2 pg/mg protein).

3.3. Ovarian blood flow

All dogs tolerated the ultrasonographic procedure well without any apparent sign of discomfort or stress. No difference in blood flow parameters was observed between right and left ovaries of each bitch throughout the diestrus. The intra-ovarian arterial blood flow showed a systolic waveform characterized by steep increase and decrease and a slow diastolic wave with relatively high-end diastolic velocity (Fig. 2, upper). From day 9 to 36 of the estrous cycle, the calculated blood flow parameters, color-coded area, and SPV decreased progressively, but similarly in both control and treated dogs (Fig. 3, panels A and C, respectively), whereas the RI and EDV remained almost unchanged (Fig. 3, panels B and D, respectively). From day 38, the differential decreases of the vascularized area, SPV, and EDV variables were greater ($P < 0.01$) in aglepristone-treated than in control bitches, whereas the RI had an opposite trend and increased ($P < 0.01$) thereafter (Fig. 3B). All the blood flow parameters were undetectable at 60 ± 3.6 (n = 4) and 68 ± 2.0 (n = 4) days in treated and control bitches, respectively.

In control bitches, functional luteal regression, expressed by serum progesterone level decrease, was accompanied by a parallel decline in ovarian blood supply (days 29 to 72, $r = 0.791$, n = 60, $P < 0.001$) with a shift of approximately 8 to 10 days (Fig. 4). In treated bitches, both progesterone and ovarian blood flow curves exhibited a similar tendency (days 29 to 72, $r = 0.808$, n = 60, $P < 0.001$), but were much steeper (Coincidence test: $F = 31.854$, df = numerator 2 and denominator 116, $P < 0.001$; comparison between slopes: $t = 2.882$, df = 116, $P < 0.001$) than those of control animals (Fig. 4).

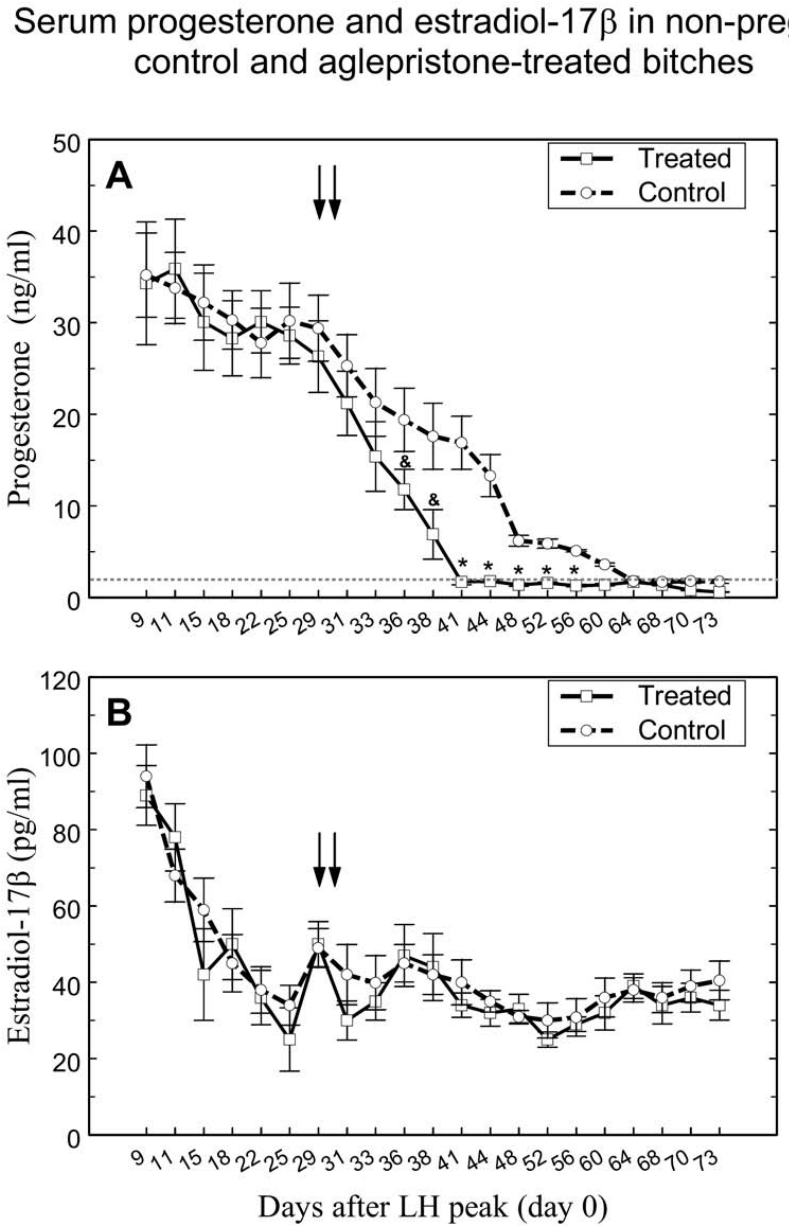

Fig. 1. Serum progesterone (panel A) and estradiol-17β (panel B) concentrations in treated (n = 9) and control (n = 9) bitches during the luteal phase, from days 9 to 73 after the estimated LH peak. The arrows point to the days of treatment with aglepristone (10 mg/kg bw) or saline. Results are expressed as means ± SD: & and asterisks indicate a significantly different values from controls ($P < 0.05$ and $P < 0.01$, respectively). The dotted line shows the limit of complete luteolysis, arbitrarily set at 2 ng/mL of progesterone.

4. Discussion

The progesterone antagonist, aglepristone, is currently used in clinical practice for induction of abortion or parturition in dogs as well as for treatment of gynecological disorders and pyometra [25–44]. However, aglepristone can also be prescribed for treatment of other pathologies caused by a relatively long exposure to high progesterone levels, including cystic endometrial hyperplasia [45], acromegaly, insulin resistance [46], mammary GH-induced IGF-I secretion [47].

In the first part of luteal phase, peripheral blood progesterone concentrations were high and similar to those found by Romagnoli et al [48] in bitches 8 to 19 days after the onset of cytological diestrus. The gradual decline of progesterone observed in dogs treated with aglepristone parallels that of controls, suggesting that this antiprogestagen triggers an anticipated, but physiological-like, luteolytic process. In the present study, luteolysis began seven days after aglepristone administration. Our results, however, are opposite to those reported by Galac et al [38] who did not find any

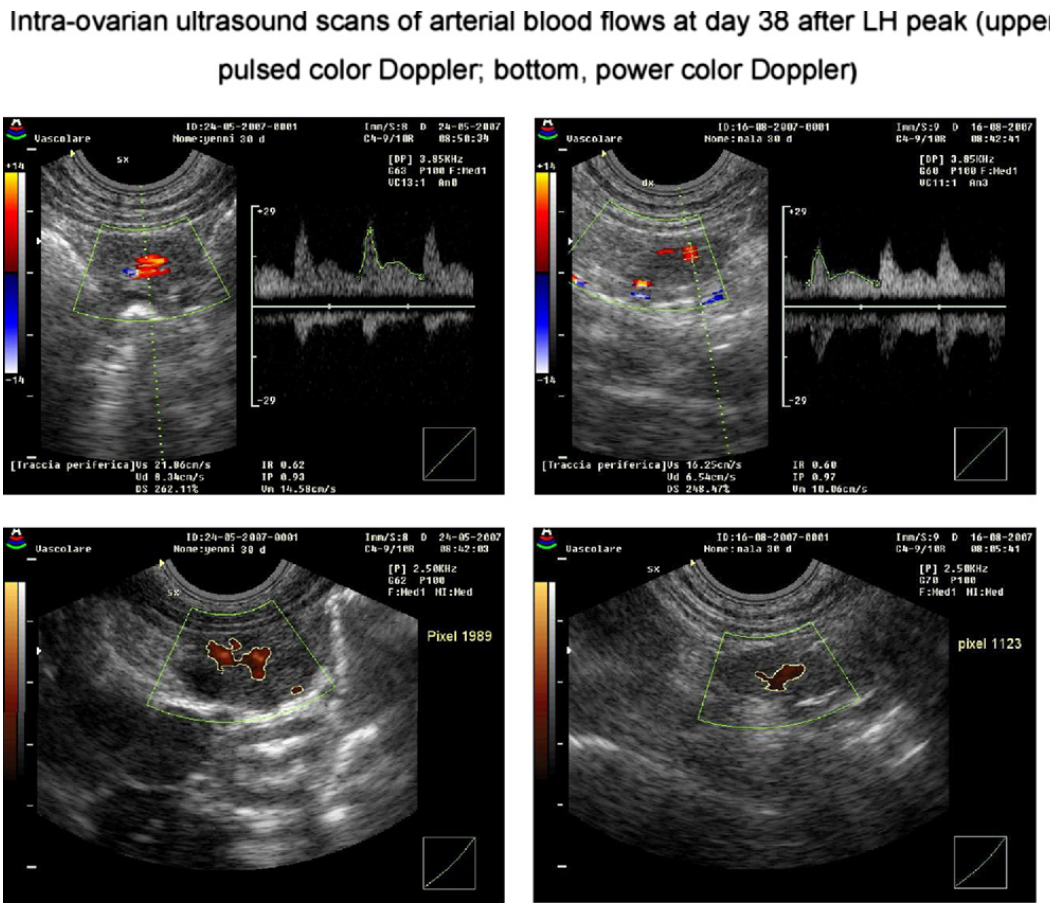

Fig. 2. Representative images of intra-ovarian arterial typical waveforms visualized by trans-abdominal scanning with color-pulsed Doppler in a representative control (upper left) and an aglepristone-treated dog (upper right) at day 38 after the estimated LH peak. Respective intra-ovarian power Doppler images mapping the sampled arterial vascularization area in a control (bottom left) and an aglepristone-treated bitch (bottom right). Aglepristone (10 mg/kg bw) or saline were administered at days 29 and 30 after the LH peak.

change in the length of the luteal phase following administration of aglepristone. This discrepancy is likely due to the different protocol applied, because the study of Galac et al [38] dosed the antiprogestagen 12 and 13 days after ovulation, while our study gave it at days 29 and 30. Thus, whereas the trial of Galac et al [38] was entirely carried out during the pituitary-independent luteal phase, in the present work the treatment overlapped the pituitary-dependent luteal phase [9,11,12,49].

Although the effects of aglepristone on luteal function of the dog can be easily traced by assessing peripheral plasma progesterone concentrations, our knowledge of the underlying neuro-endocrine mechanisms responsible for the accelerated demise of luteal function is still incomplete. Progesterone antagonists may involve hypothalamic neurons secreting GnRH, pituitary gonadotrophe as well as steroidogenic ovarian cells [50]. From a functional point of view, treatment with aglepristone simulates selective, but transitory ablation of CL that, based on current pharmacological knowledge, persists for about 6 to 8 days [51]. Thus, despite the peripheral serum progesterone levels continue to remain relatively high from day 30 onward, the specific genomic action of progesterone is competitively inhibited during this interval.

In the bitch, PR has been precisely localized in all the ovarian structures examined, including follicular and theca cells, and stromal luteal cells [12,52]. Thus, direct intra ovarian effects induced by antiprogestagen treatment cannot be ruled out. However, PR are likely expressed also in the anterior pituitary and several brain areas including the hypothalamus as well as in the endometrium. Thus an indirect action on the ovary by the aglepristone through modulation of the hypothalamic-pituitary axis cannot be excluded [22].

The serum estradiol-17β levels were high throughout the luteal phase here examined, especially during the first ten days, and comparable with those reported in previous studies in non-pregnant bitches during the mid- and late-diestrus phases [19,20,53]. Treatment with aglepristone did not modify the hormonal produc-

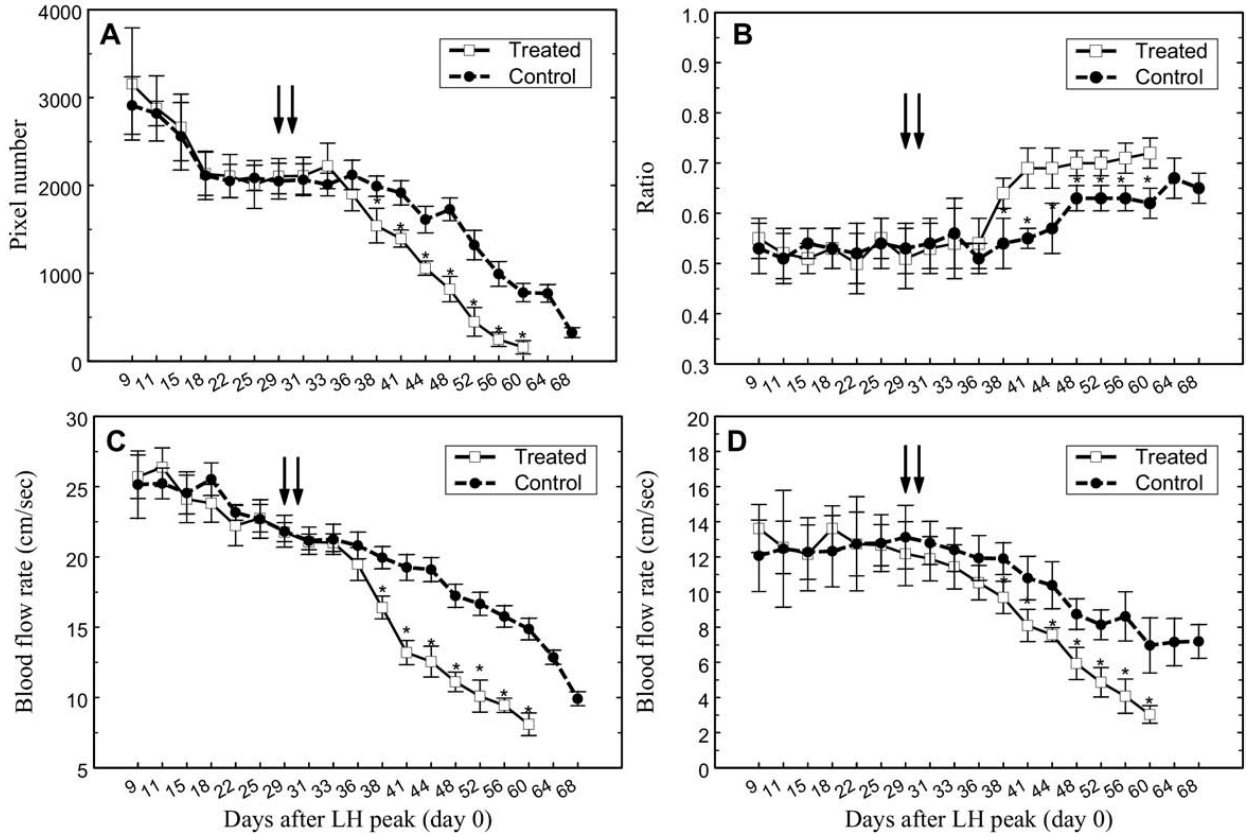

Fig. 3. In panel A, power Doppler values (expressed in number of pixel) calculated from right and left intra-ovarian arterial blood flows in control (n = 4) and aglepristone-treated (n = 4) bitches from days 9 to 68 after the estimated LH peak. In panel B, C, and D values of resistance index (RI), systolic peak velocity (SPV, cm/sec), and end-diastolic velocity (EDV, cm/sec) respectively, of intra-ovarian arterial blood flow. Values are medians ± SD and those with an asterisk are significantly different ($P < 0.05$) from controls. The arrows point to the days of treatment (29 and 30 after LH peak) with aglepristone (10 mg/kg bw) or saline.

tion of estradiol-17β. In fact, the secretory pattern of this steroid remained unchanged and super-imposable to that of control dogs. Moreover, no changes in estradiol-17β peripheral concentrations were observed in concurrence with the regression of luteal function in either control or aglepristone-treated bitches in the late luteal phase, as previously reported by Onclin et al [19]. Our data from the *in vitro* study clearly demonstrate that the CL of the bitch, explanted at day 38 after LH peak, synthesize not only progesterone, but also substantial amounts of estradiol-17β, thus confirming previous observation that the luteal tissue of the dog has the enzymatic machinery to synthesize and release estrogens [22]. However, the question whether estradiol-17β is part of a luteotrophic complex and has a role in luteal regression as in other species remains unanswered.

The CL have the highest blood flow rates of any tissue or organ [54,55]. Thus, local changes in the rate of ovarian perfusion may affect the secretory function of CL, given that maximum perfusion of the ovary reflects the period of highest progesterone production. Only recently has the development of color Doppler ultrasonographic technique made real-time study of blood flow parameters feasible. In bitches, Doppler ultrasound has been used to assess maternal and fetal blood flow during gestation [39,56] and ovarian perfusion changes during estrus cycle [57]. High blood flow velocities and low PI and RI characterized the early-diestrus phases in bitches, in good agreement with the previous ultrasonographic study of ovarian perfusion during the normal estrus cycle [57]. In the early luteal phase, peripheral progesterone concentrations were also at their highest levels.

Throughout the second part of luteal phase (>day 31), the relative decrease of blood progesterone was highly and positively correlated with the concurrent decrease of the blood flow supplying the ovaries, as assessed by power Doppler ultrasound. In our model, however, the ovarian blood flow curves were shifted by approximately 8–10 days beyond those of progesterone production. Thus, the hypothesis that progesterone de-

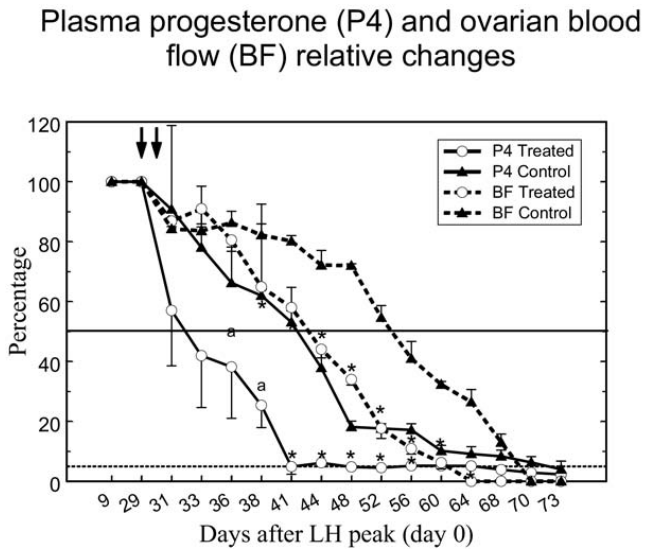

Fig. 4. Relative changes of serum progesterone (P4, continuous lines) and ovarian blood flow (OBF, dotted lines) in control (triangles) and aglepristone-treated bitches (circles). The progesterone and hemodynamic data are expressed as a percentage of respective values for the first 29 days after the estimated LH peak (100% = 30.46 ± 6.85 and 28.77 ± 8.52 ng/mL for progesterone in control and treated dogs, respectively; 100% = 2366 ± 381 and 2446 ± 444 pixel number for blood flow in control and treated dogs, respectively). The arrow points to the days of treatment with aglepristone (10 mg/kg bw) or saline. The continuous line shows the limit of partial luteolysis, arbitrarily defined as a 50% decline in progesterone concentration, whereas the dotted line shows the limit of complete luteolysis, which was arbitrarily set at 2 ng/mL of progesterone. Each point represents the median ± SD of four values; (a) $P < 0.05$ and (*) $P < 0.01$ vs. control.

cline is responsible for the decrease of blood flow is more convincing than the opposite claiming that the reduced supply of nutrients carried to the ovary by the blood is the indirect cause of reduced steroidogenesis. Taken together, these findings suggest that the ovarian blood flow of dogs is regulated by several intraovarian factors, including progesterone. Receptors for progesterone and estradiol-17β have been found in several components (muscular layer) of the arterial vessel [58,59]. Thus, progesterone receptors are likely involved in the modulation of vessel resistance, and therefore in ovarian blood flow, through local modulation in the expression of vasoactive peptides such as endothelin-1 and angiotensin-II, as well as intraluteal synthesis of PGF2α, which causes vasoconstriction [60–61].

In conclusion, the present study indicates that aglepristone administration to dogs during the mid-luteal phase markedly reduces the length of progesterone secretion by CL and accelerates the luteolytic process. Collectively, our data do not support the hypothesis of aglepristone-induced ovarian blood flow reduction preceding luteolysis. However, it remains to be better defined whether the luteolytic effect of aglepristone is mediated by suppression of the luteotrophic support of pituitary gonadotropins or by inhibition of the positive effects of progesterone acting as an autocrine factor with positive feedback action on steroidogenesis.

Acknowledgements

This work was partly supported by a grant from Ministero dell'Istruzione, dell'Università e della Ricerca (Contract no. 2005078028). The authors gratefully acknowledge the generous gift of Alizine by Virbac (France). The authors wish to thank Sheila Beatty for the revision of the English text.

References

[1] Heap RB, Galil AK, Harrison FA, Jenkin G, Perry JS. Progesterone and oestrogen in pregnancy and parturition: comparative aspects and hierarchical control. Ciba Found Symp 1977;47:127–57.
[2] Spencer TE, Bazer FW. Biology of progesterone action during pregnancy recognition and maintenance of pregnancy. J Ovarian Res 2002;7:1879–98.
[3] Spencer TE, Johnson GA, Burghardt RC, Bazer FW. Progesterone and Placental Hormone Actions on the Uterus: Insights from Domestic Animals. Biol Reprod 2004;71:2–10.
[4] Chakraborty PK. Reproductive hormone concentrations during estrus, pregnancy, and pseudopregnancy in the Labrador bitch. Theriogenology 1987;27:827–40.
[5] Gobello C, Concannon PW, Verstegen J. Canine peudopregnancy: a review. In: P. Concannon, G. England, J. Verstegen, editors. Recent advances in small animal reproduction. International Veterinary Information Service. 2002. (www.ivis.org).
[6] Marinelli L, Rota A, Carnier P, Da Dalt L, Gabai G. Factors affecting progesterone production in corpora lutea from pregnant and diestrus bitches. Anim Reprod Sci 2009;114:289–300.
[7] Verstegen-Onclin K, Verstegen J. Endocrinology of pregnancy in the dog: A review. Theriogenology 2008;70:291–9.
[8] Concannon PW. Effects of hypophysectomy and of LH administration on luteal phase plasma progesterone levels in the Beagle bitch. J Reprod Fertil 1980;58:407–10.
[9] Concannon PW, Weinstein P, Whaley S, Frank D. Suppression of luteal function in dogs by luteinizing hormone antiserum and by bromocryptine. J Reprod Fertil 1987;81:175–80.
[10] Okkens AC, Dieleman SJ, Bevers MM, Lubberink AA, Willemse AH. Influence of hypophysectomy on the life span of the corpus luteum in the cyclic dog. J Reprod Fertil 1986;77:187–92.
[11] Okkens AC, Bevers MM, Dieleman SJ, Willemse AH. Evidence for prolactin as the main luteotrophic factor in the cycling dog. Vet Q 1990;12:193–201.
[12] Hoffmann B, Busges F, Engel E, Kovalevski MP, Papa P. Regulation of corpus luteum function in the bitch. Reprod Domest Anim 2004;39:232–40.

[13] Concannon PW. Endocrinologic control of normal canine ovarian function. Reprod Domest Anim 2009;44 Suppl 2:3–15.
[14] Fernandes PA, Bowen RA, Kostas AC, Sawyer HR, Nett TM, Olson PN. Luteal function in the bitch: changes during diestrus in pituitary concentration of and the number of luteal receptors for luteinizing hormone and prolactin. Biol Reprod 1987;37: 804–11.
[15] de Gier J, Kooistra HS, Djajadiningrat-Laanen SC, Dieleman SJ, Okkens AC. Temporal relations between plasma concentrations of luteinizing hormone, follicle-stimulating hormone, estradiol-17beta, progesterone, prolactin, and alpha-melanocyte-stimulating hormone during the follicular, ovulatory, and early luteal phase in the bitch. Theriogenology 2006;65:1346–59.
[16] Kooistra HS, Okkens AC. Secretion of growth hormone and prolactin during progression of the luteal phase in healthy dogs: a review. Mol Cell Endocrinol 2002;197:167–72.
[17] Concannon PW, Cowan R, Hansel W. LH release in ovariectomized dogs in response to estrogen withdrawal and its facilitation by progesterone. Biol Reprod 1979;20:523–31.
[18] Peluso JJ. Multiplicity of progesterone's actions and receptors in the mammalian ovary. Biol Reprod 2006;75:2–8.
[19] Hoffmann B, Höveler R, Nohr B, Hasan SH. Investigations on hormonal changes around parturition in the dog and the occurrence of pregnancy-specific non conjugated oestrogens. Exp Clin Endocrinol 1994;102:185–9.
[20] Onclin K, Murphy B, Verstegen JP. Comparison of estradiol, LH and FSH patterns in pregnant and non pregnant beagle bitches. Theriogenology 2002;57:1957–72.
[21] Luz MR, Bertan CM, Binelli M, Lopes MD. Plasma concentrations of 13,14-dihydro-15-keto prostaglandin F2-alpha (PGFM), progesterone and estradiol in pregnant and nonpregnant diestrus crossbred bitches. Theriogenology 2006;66:1436–41.
[22] Nishiyama T, Tsumagari S, Ito M, Kimura J, Watanabe G, Taya K, Takeishi M. Immunohistochemical study of steroidogenic enzymes in the ovary and placenta during pregnancy in the dog. Anat Histol Embryol 1999;28:125–9.
[23] Cadepond F, Ulmann A, Baulieu EE. RU486 (mifepristone): mechanisms of action and clinical uses. Annu Rev Med 1997; 48:129–56.
[24] Mahajan DK, London SN. Mifepristone (RU486): a review. Fertil Steril 1997;68:967–76.
[25] Hoffman B, Schuler H. Receptor blockers – general aspects with respect to their use in domestic animal reproduction. Anim Reprod Sci 2000;60–61:295–312.
[26] Psychoyos A, Nikas G, Sarantis I, Gravantis A. Hormonal anti-implantation agents: anti-progestins. Hum Reprod 1995;10: 140–50.
[27] Sarkar NN. The potential of mifepristone (RU-486) as an emergency contraceptive drug. Acta Obstet Gynecol Scand 2005;84: 309–16.
[28] Ozalp GR, Calışkan C, Seyrek-İntaş K, Wehrend A. Effects of the progesterone receptor antagonist aglepristone on implantation administered on days 6 and 7 after mating in rabbits. Reprod Domest Anim 2008 Nov 18. [Epub ahead of print].
[29] Zhu HX, Zhang WW, Zhuang YL, Huang LL. Mifepristone as an anti-implantation contraceptive drug: roles in regulation of uterine natural killer cells during implantation phase. Am J Reprod Immunol 2009;61:68–74.
[30] van der Weyden GC, Taverne MAM, Dieleman SJ, Wurth Y, Bevers MM, van Oord HA. Physiological aspects of pregnancy and parturition in dogs. J Reprod Fertil Suppl 1989;39:211–24.
[31] Concannon PW, Yeager A, Franck D, Iyampillai A. Termination of pregnancy and induction of premature luteolysis by the antiprogestagen, mifepristone, in dog. J Reprod Fertil 1990;88: 99–104.
[32] Linde-Forsberg C, Kindahl H, Madej A. Termination of midterm pregnancy in the dog with oral RU 486. J Small Anim Pract 1992;33:331–6.
[33] Fieni F, Marnet PG, Martal J, Siliart B, Touzeau N, Bruyas JF, Tainturier D. Comparison of two protocols with a progesterone antagonist aglepristone (RU534) to induce parturition in bitches. J Reprod Fertil 2001;57(Suppl.):237–42.
[34] Fieni F, Martal J, Marnet PG, Siliart B, Bernard F, Riou M, Bruyas JF, Tainturier D. Hormonal variation after early or mid-pregnancy termination in bitches with aglepristone (RU534). J Reprod Fertil 2001;57(Suppl):243–8.
[35] Baan M, Taverne MAM, Kooistra HS, de Gier J, Dieleman SJ, Okkens AC. Induction of parturition in the bitch with the progesterone-receptor blocker aglepristone. Theriogenology 2005; 63:1958–72.
[36] Galac S, Kooistra HS, Butinar J, Bevers MM, Dieleman SJ, Voorhout G, Okkens AC. Termination of mid-gestation pregnancy in bitches with aglepristone, a progesterone receptor antagonist. Theriogenology 2000;53(4):941–50.
[37] Gerres S, Hoffmann B. Investigations on the role of progesterone in the endocrine control of overt pseudopregnancy in the bitch: application of an antigestagen and effects on corpus luteum function. Anim Reprod Sci 1994;35:281–89.
[38] Galac S, Kooistra HS, Dieleman SJ, Cestnik V, Okkens AC. Effects of aglépristone, a progesterone receptor antagonist, administered during the early luteal phase in non-pregnant bitches. Theriogenology 2004;62:494–500.
[39] Di Salvo P, Bocci F, Zelli R, Polisca A. Doppler evaluation of maternal and foetal vessels during normal gestation in the bitch. Res Vet Sci 2006;81:382–8.
[40] Holst PA, Phemister RD. Temporal sequence of events in the estrous cycle of the bitches. Am J Vet Res 1975;36:705–6.
[41] Zerani M, Dall'Aglio C, Maranesi M, Gobbetti A, Brecchia G, Mercati F, Boiti C. Intraluteal regulation of prostaglandin F2α-induced prostaglandin biosynthesis in pseudopregnant rabbits. Reproduction 2007;133:1005–16.
[42] Gobbetti A, Zerani M, Bellini-Cardellini L. Relationships among mammalian gonadotropin-releasing hormone, prostaglandines and sex steroids in the brain of the crested newt, Triturus cornifex. Prostaglandins 1992;44:209–18.
[43] Scotti L, Di Salvo P, Bocci F, Pieramati C, Polisca A. Doppler evaluation of maternal and foetal vessels during normal gestation in queen. Theriogenology 2008;69:1111–9.
[44] Gobello C. Dopamine agonists, anti-progestins, anti-androgens, long-term-release GnRH agonists and anti-estrogens in canine reproduction: a review. Theriogenology 2006;66:1560–7.
[45] Bhatti SF, Rao NA, Okkens AC, Mol JA, Duchateau L, Ducatelle R, van den Ingh TS, Tshamala M, Van Ham LM, Coryn M, Rijnberk A, Kooistra HS. Role of progestin-induced mammary-derived growth hormone in the pathogenesis of cystic endometrial hyperplasia in the bitch. Domest Anim Endocrinol 2007;33:294–312.
[46] Lee WM, Kooistra HS, Mol JA, Dieleman SJ, Schaefers-Okkens AC. Ovariectomy during the luteal phase influences secretion of prolactin, growth hormone, and insulin-like growth factor-I in the bitch. Theriogenology 2006;66:484–90.
[47] Beijerink NJ, Bhatti SF, Okkens AC, Dieleman SJ, Duchtateau L, Van Ham LM, Kooistra HS. Adenohypophyseal function in

bitches treated with medroxyprogesterone acetate. Domest Anim Endocrinol 2007;32:63–78.
[48] Romagnoli SE, Camillo F, Novellini S, Johnston SD, Cela M. Luteolytic effects of prostaglandin F2α on day 8 to 19 corpora lutea in the bitch. Theriogenology 1996;45:397–403.
[49] Vickery BH, Bergstrom K, Killer MB, Goodpasture JC. Effect of Detirelix a long acting LHRH antagonist on luteal function and pregnancy in beagle bitches. 1987;36(Suppl):297. abstract.
[50] Rothchild I. The corpus luteum revisited: are the paradoxical effects of RU486 a clue to how progesterone stimulates its own secretion? Biol Reprod 1996;55:1–4.
[51] Fieni F, Tainturier D, Bruyas JF, Badinand F, Lefay MP. Etude clinique d'une anti-hormone pour provoquer l'avortement chez la chienne: l'aglepristone. Rec Med Vet 1996;144:285–9.
[52] Vermeirsch H, Simoens P, Coryn M, Van den Broeck W. Immunolocalization of progesterone receptors in the canine ovary and their relation to sex steroid hormone concentrations. Reproduction 2001;122:73–83.
[53] Graf KJ. Serum oestrogen, progesterone and prolactin concentrations in cyclic, pregnant and lactating beagle dogs. J Reprod Fertil 1978;52:9–14.
[54] Sugino N, Matsuoka A, Taniguchi K, Tamura H. Angiogenesis in the human corpus luteum. Reprod Med Biol 2008;7:91–103.
[55] Takasaki A, Tamura H, Taniguchi K, Asada H, Taketani T, Matsuoka A, Yamagata Y, Shimamura K, Morioka H, Sugino N. Luteal blood flow and luteal function. J Ovarian Res 2009;2:1.
[56] Blanco PG, Arias DO, Gobello C. Doppler ultrasound in canine pregnancy. J Ultrasound Med 2008;27:1745–50.
[57] Köster K, Poulsen Nautrup C, Günzel-Apel AR. A Doppler ultrasonographic study of cyclic changes of ovarian perfusion in the Beagle bitch. Reproduction 2001;122:453–61.
[58] Qiao X, McConnell KR, Khalil RA. Sex steroids and vascular responses in hypertension and aging. Gend Med 2008;5(Suppl A):46–64.
[59] Chennazhi KP, Nayak NR. Regulation of angiogenesis in the primate endometrium: vascular endothelial growth factor. Semin Reprod Med 2009;27:80–9.
[60] Acosta TJ, Yoshizawa N, Ohtani M, Miyamoto A. Local changes in blood flow within the early and midcycle corpus luteum after prostaglandin F2α injection in the cow. Biol Reprod 2002;66:651–58.
[61] Miyamoto A, Shirasuna K, Wijayagunawardane MP, Watanabe S, Hayashi M, Yamamoto D, Matsui M, Acosta TJ. Blood flow: a key regulatory component of corpus luteum function in the cow. Domest Anim Endocrinol 2005;29:329–39.