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Investigation of the Relationship between Endometrial MMP Activities and Sex Hormones in Mature Bitches

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Original Article

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Abstract

Endometrial matrix metalloproteinases (MMPs) are extensively studied in humans. However, owing to the distinct differences of the estrous cycle of bitches, the expression and activities of endometrial MMPs regulated by sex hormones should be significantly different. To understand the effect of sex hormones on the activity of MMPs, this study investigates the activities of endometrial MMP-2 and MMP-9 during different phases of the estrous cycle in bitches through the use of gelatine zymography. Estradiol and progesterone concentrations were also determined using enzyme-linked fluorescent assay. The activities of the latent forms of both MMP-2 and -9 were significantly higher in metestrus (MP) and late diestrus phases (L-DP) than in anestrus (AP) and proestrus/estrus phases (EP) (p<0.05). Similarly, the activities of the active forms of both MMPs in L-DP were significantly higher than in the other phases of the estrous cycle (p<0.05). Significant correlations were found between progesterone and the activities of latent and active forms of MMP-9 during MP (r= -0.83, p<0.05 and r= 0.94, p<0.01, respectively). Considering our results, together with that of previous studies on the role of MMPs in the reproductive system, we hypothesize that endometrial MMP-2 and MMP-9 play a key role in successful fertilization.

Keywords: dogs, endometrium, estrous cycle, matrix metalloproteinase

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บทคัดย่อ

การศึกษาความสัมพันธ์ระหว่างค่าของ MMP ของเยื่อบุผนังมดลูกและฮอร์โมนเพศในสุนัขเพศเมียวัยเจริญพันธุ์

Wei-Yau Shia[1] Po-Chen Chu[1] Chih-Chung Chou[1, 2] Wei-Ming Lee[1,2]*

การแสดงออกของโปรตีน matrix metalloproteinases (MMPs) ในเยื่อบุผนังมดลูกในคนได้มีการศึกษาอย่างกว้างขวาง อย่างไรก็ตาม ยังคงพบความแตกต่างในแต่ละรอบวงจรการเป็นสัดในสุนัข ซึ่งการแสดงออกของโปรตีนดังกล่าวจะถูกควบคุมโดยฮอร์โมนเพศ วัตถุประสงค์ของการศึกษาครั้งนี้ คือ ศึกษาผลของฮอร์โมนเพศที่มีต่อการแสดงออกของโปรตีน MMPs, ทำการตรวจวัดค่า MMP-2 และ MMP-9 ของเยื่อบุผนังมดลูก ในแต่ละช่วงของวงจรการเป็นสัดโดยวิธี gelatine zymography ร่วมกับการตรวจระดับฮอร์โมนเอสตราไดออลและโปรเจสเตอโรน โดยวิธี enzyme-linked fluorescent assay ค่าของ latent form MMP-2 และ MMP-9 ในระยะ metestrus (MP) และ diestrus ระยะท้าย สูงกว่าระยะ anestrus (AP) และ proestrus และ estrus phases (EP) อย่างมีนัยสำคัญทางสถิติ ($p<0.05$). ซึ่งคล้ายคลึงกับค่าของ active forms MMPs ในระยะ L-DP มีค่าสูงกว่าในระยะอื่นของวงจรการเป็นสัด อย่างมีนัยสำคัญทางสถิติ ($p<0.05$) นอกจากนี้ยังพบความสัมพันธ์ทางสถิติระหว่างระดับฮอร์โมนโปรเจสเตอโรน และค่าของ latent และ active forms MMP-9 ในช่วง MP ($r= -0.83$, $p<0.05$ และ $r= 0.94$, $p<0.01$, ตามลำดับ). ผลการศึกษาในครั้งนี้สอดคล้องการศึกษาที่ผ่านมา ในด้านบทบาทของ MMPs ต่อระบบสืบพันธุ์ โดยมีสมมุติฐานว่าการแสดงออกของ MMP-2 และ MMP-9 ในเยื่อบุผนังมดลูกมีบทบาทที่สำคัญในขบวณการปฏิสนธิ.

คำสำคัญ: สุนัข เยื่อบุผนังมดลูก วงรอบการเป็นสัด matrix metalloproteinase

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Introduction

Endometrium is a tissue rich in blood vessels and contains special stroma and glands. Dramatic changes in the endometrium during postpartum involution, cystic endometrial hyperplasia, and pyometra are well known in the canine uterus (Al-Bassam et al., 1981; Dhaliwal et al., 1998). However, during the normal estrous cycle, cytology and morphology of endometrial cells and surface structure undergo changes during different stages of the estrous cycle (Van Cruchten et al., 2003; Groppetti et al., 2010). These results reveal the micro-environmental changes of the endometrium during the estrous cycle in bitches. Matrix metalloproteinases (MMPs) are found to participate in the process of changes in the endometrium in both humans and dogs (Marbaix et al., 1995; Irwin et al., 1996; Salamonsen and Woolley, 1999; Chu Py et al., 2002).

MMPs are a group of enzymes capable of digesting extracellular matrix. Their concentration and activity increase during normal tissue remodeling (such as embryo development and skeletal remodeling) or inflammation caused by some diseases (such as gingivitis and rheumatoid arthritis) (Woessner, 1991). Although there are different substrate specificities possessed by the members of MMPs, there is homology of the sequences which define the shared domains: a signal sequence, a pro-domain, a zinc-binding domain containing the catalytic site, and a hemopexin domain (Page-McCaw et al., 2007). The pro-domain has a conserved Cys residue which coordinates the active-site zinc to inhibit catalysis. Activation of MMPs is through removal of the pro-domain to enable the active-site to catalyze substrates (Vu and Werb, 1998; Yu et al., 1998; Page-McCaw et al., 2007). Through *in situ* hybridization and immunohistochemical staining of human endometrium, only MMP-7 is found in glandular epithelium. The other MMPs are all localized in stromal cells (MMP-1, 2, 3, 10, and 11) and inflammatory cells (MMP-9) (Rodgers et al., 1993; Rodgers et al., 1994; Marbaix et al., 1995; Jeziorska et al., 1996; Salamonsen and Woolley, 1996; Zhang and Salamonsen, 1997). During the menstrual cycle, only MMP-2 and the tissue inhibitor of metalloproteinase-1 (TIMP-1) can be detected during the reproductive cycle. MMP-3 and MMP-9 are only found in late-secretory phase and MMP-7 and MMP-11 are only found in the proliferative phase (Rodgers et al., 1994; Salamonsen and Woolley, 1999). These results indicate that MMPs have cytological and reproductive cycle specificity and also play a pivotal role in

endometrial growth and remodeling during the menstrual cycle. In comparison with MMPs in the human menstrual cycle, no significant differences are found in endometrium MMPs during artificially simulated (exogenous oestradiol and progesterone) estrous cycles in dogs (Chu Py et al., 2002). However, the levels of MMPs in the endometrium of intact bitches are unknown. Additionally, MMPs do not only exist in the female reproductive system, but can also be found in males. MMP-2 and MMP-9 are the predominant MMPs in human seminal plasma and sperm (Buchman-Shaked et al., 2002; Shimokawa Ki et al., 2002; Shimokawa et al., 2003; Tentes et al., 2007). Furthermore, the concentration of MMP-2 is strongly correlated to the sperm count (Baumgart et al., 2002), but higher MMP-9 is associated with abnormal sperm samples (Buchman-Shaked et al., 2002; Tentes et al., 2007).

When considering the effect of sex hormones on MMP expression in the endometrium, MMP 1, 2, 3 and 9 are reported to increase in response to progesterone withdrawal in human endometrial stromal cells and/or endometrial tissue explant studies (Marbaix et al., 1992; Salamonsen et al., 1997; Lockwood et al., 1998). MMP expression in the endometrium is at its lowest level with the endometrium exposed to maximum concentrations of progesterone, but increases during the immediate premenstrual and menstrual phases as progesterone support falls (Marbaix et al., 1992; Salamonsen et al., 1997; Lockwood et al., 1998). As we know, the release of sex hormones in bitches is similar to that in humans. However, the estrous cycle in bitches differs with the menstrual cycle in that the endometrium is shed during menstruation. This significant physical difference should cause a dramatic difference in MMPs expression, in the canine endometrium. Chu and colleagues evaluated the effect of exogenous sex hormone on MMPs expression but found no significant difference (Chu Py et al., 2002). Information about the effect of sex hormones on MMPs expression in the endometrium of normal intact bitches is still limited.

The endometrium is sensitive to hormones and provides the specific environment necessary for the support of early embryo implantation and development (Concannon et al., 2001). Successful fertilization and implantation require adequate sperm quality and an appropriate endometrial environment. Within this environment, MMPs play an important role. This study investigated MMP activity in the endometrium of normal bitches through the use of gel zymography, while simultaneously evaluating the effects of sex hormones on MMP activity during different stages of the estrous cycle. Owing to the prolong time effect of progesterone on endometrium, the changes of endometrial MMPs were very likely correlated with progesterone. In contrast to the secretory length of progesterone, estrogen might not apparently correlate with endometrial MMPs during estrus cycle in bitches.

Materials and Methods

Animals: A total of 64 intact bitches of normal health were submitted for ovariohysterectomy surgery at the Veterinary Medicine Teaching Hospital, at National Chung Hsing University, Taichung, Taiwan. The mean age was 24.4±3.4 months. Each phase of the estrous cycle was diagnosed clinically by the behavior, appearance of the mammary glands and pudendum, and vaginal excretions. Ovarian and uterine tissues were collected for histological examination in order to classify the specific stages of the cycle (Rehm et al., 2007). Through the use of progesterone concentrations to determine and differentiate the different phases of the estrous cycle (Van Cruchten et al., 2004), four phases were separated and modified in our study: anestrus phase (AP) (n=12), proestrus/estrus phase (EP) (n=26), metestrus phase (MP) (n=6), and late diestrus phase (L-DP) (n=20). Briefly, the histological characteristics of AP included a regressing corpus luteum, atrophic endometrium and a progesterone level lower than 1 ng/ml. The EP contained a developing corpus luteum, proliferating surface of endometrial epithelium, and progesterone levels between 1 and 15 ng/ml. Dogs with a developing or fully developed corpus luteum, proliferative endometrium, and progesterone levels higher than 15 ng/ml, were assigned to MP. L-DP showed a regressed corpus luteum, endometrial apoptosis, and progesterone levels lower than 10 ng/ml and higher than 0.5 ng/ml. The samples used in this study were obtained with the approval of the animal owner.

Anesthetic protocols: General anesthesia in all bitches was induced with intravenously administered propofol (Lipuro®, B. Braun Melsungen, Germany) and maintained by inhalation anaesthesia with isoflurane (IsoFlo®, Abbott, Farum, USA) and O2. Intravenous fluid therapy (dextrose-lactated Ringer's solution, Taiyu, Taiwan) was administered to all bitches during ovariohysterectomy.

Sample preparation: Blood samples were collected from the jugular vein before surgery and stored in heparin-coated tubes (Vacuette®, Cen-Med Enterices, USA). After centrifugation, plasma samples for the measurements of estrogen and progesterone were collected and stored at -70°C. Endometrial tissues were carefully scraped out using a No. 15 surgical blade, taking care not to include myometrial tissue from the uterus. When the color of the scraped tissue was getting whiter, meaning that myometrium might be involved, the scraping was stopped. The 50 mg samples of endometrial tissue were placed in at least three anti-freeze tissue storing tubes (Nalgene®, Cryoware™, NY, USA) and immediately stored at -70°C until the gelatin zymography assay. For detection of MMP activity, 1 tube of endometrial tissue was mixed with 1 ml PBS (containing 137 mmol/l NaCl, 2.7 mmol/l KCl, 10 mmol/l Na_2HPO_4, and 2 mmol/l KH_2PO_4) and ground in a glass tissue grinder (OMNI International, GA, USA) to make an emulsion. All procedures were conducted on ice to prevent protein degradation due to heat production. The grinder was washed with detergent and 95% alcohol several times to remove residue proteins after each tissue grinding. The emulsion was centrifuged at

13,000 g for 30 min at 4ºC. Supernatant was used for detecting MMPs by gel zymography.

Hormone determination: Plasma estradiol and progesterone were detected by enzyme-linked fluorescent assay (VIDAS® Estradiol and VIDAS® Progesterone, bioMerieux SA, France). Manipulation procedures were carried out according to the manufacturer's instructions. Before detection, all strips and special tips were warmed at room temperature for at least 30 min. 200 µl of plasma were used for detecting each hormone. All loading and reacting procedures were automatically manipulated (miniVIDAS®, bioMerieux SA, France). The determining limits of estradiol and progesterone were 9-3000 pg/ml and 0.25-85 ng/ml, respectively.

Gelatin zymography: Proteases were detected by their capacity to degrade specific substrates as previously described (Leber and Balkwill, 1997; Shia et al., 2011). Briefly, 10 µl of sample (supernatant of endometrial emulsion) and 2 µl of human MMP-2 marker (Chemicon®, CA) diluted with an equal volume of sample buffer (containing 0.125 M Tris-HCl, 20% glycerol, 4% sodium dodecyl sulfate, and trace of bromophenol blue) were loaded onto a 3% acrylamide stacking gel /6% separating acrylamide gel containing 0.2% gelatin, in the presence of sodium dodecyl sulfate (SDS), and subjected to electrophoresis. Gels were then washed twice with 2.5% Triton X-100, rinsed with distilled water and incubated at 37ºC overnight in an activation buffer containing 50mM Tris-HCl, 5 mM $CaCl_2$ at pH 7.6. After incubation, the gels were stained with Coomassie Brillant Blue and destained in a solution of 25% ethanol and 10% acetic acid. Zones of enzymatic activity were revealed by negative staining. Proteolytic areas appeared as clear bands against a dark background. Molecular weights of gelatinolytic bands were estimated using recombinant human MMP-2 and MMP-9 (R&D Systems, Minnesota, USA). Proteolytic bands in the zymogram gels were quantified using Image J Software (National Institute of Health, USA).

Statistical analysis: Statistical analysis was performed using SAS 9.0 (SAS Institute Inc., Cary, NC, USA). Normal distribution of collected data was analyzed by the Shapiro-Wilk test. In the normally distributed data, differences in MMP activities between different phases of the estrous cycle were assessed by a two-tailed Student's t-test. For the activities of endometrial MMPs which were not normally distributed, a non-parametric Mann-Whitney U test was used to compare values in different phase of the estrous cycle. For all comparisons, p value < 0.05 was considered a significant difference. All data in the study were presented as mean±SEM. Correlation coefficients between sex hormones and MMPs were calculated by Spearman's correlation coefficient.

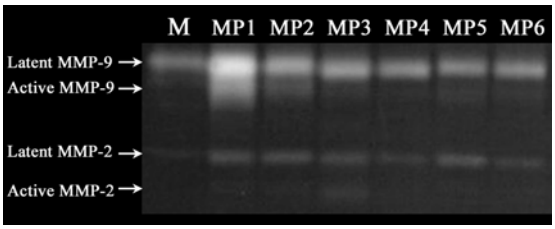

Figure 1 Electrophoresis result of gelatin zymography. Extracts from endometrium samples revealed four bands: latent MMP-9 (92 kDa), active MMP-9 (84 kDa), latent MMP-2 (kDa), and active MMP-2 (kDa). M: marker; MP: metestrus phase.

Table 1 MMP activity in different phases of the estrous cycle (Mean±SEM)

Estrous Cycle	Activity of MMPs (densitometric units)			
	MMP-9		MMP-2	
	Latent	Active	Latent	Active
Anestrus	26.1±7.1[a]	3.7±0.3[a]	64.9±15.6[a]	1.9±0.3[a]
Proestrus/Estrus	46.7±4.5[b]	7.1±0.8[b c]	91.3±20.7[a]	2.5±0.2[a]
Metestrus	235.8±25.8[c]	9.2±2.3[c d]	217.5±19.7[b c]	6.6±4.0[a b]
Late Diestrus	151.4±28.0[d]	14.6±1.8[d]	250.9±36.6[c]	6.8±1.4[b]

Results

During different phases of the estrous cycle, activities of the latent forms of MMP-2 and MMP-9 were all significantly higher than the active ones (p<0.01). The activities of MMP-2 and -9 in the endometrium of different phases of the estrous cycle are shown in table 1. Differentiation of activities of the active and latent forms of MMP-2 and MMP-9 from endometrium extracted samples were illustrated as figure 1. The activities of both latent and active forms of MMPs were all increased in MP and L-DP compared with AP and EP. The mean activities of latent and active forms of endometrial MMP-9 from MP to L-DP (170.9±23.2 and 13.3±1.5, respectively) were significantly higher than that from AP to EP (40.2±4.1 and 6.1±0.6, respectively) (p<0.01). Similarly, the mean activities of latent and active forms of endometrial MMP-2 from MP to L-DP (243.2±28.5 and 6.7±1.4, respectively) were significantly higher than that from AP to EP (83.0±15.0 and 2.3±0.2, respectively) (p<0.01). The activity of latent MMP-9 was elevated in EP (46.7±4.5) and significantly higher than that in AP (p<0.05). The activity reached the highest value during MP (235.8±25.8) and was significantly higher than all of the other phases (p<0.01 compared with AP and EP, p<0.05 compared with L-DP) (Fig 2). The activity of active MMP-9 was significantly increased in EP (7.1±0.8) (p<0.01 compared with AP) and reached highest value until L-DP (14.6±1.8). The activity of active MMP-9 in L-DP (14.6±1.8) was significantly higher than that in AP (3.7±0.3) and EP (7.1±0.8) (p<0.01) (Fig 3). Both latent

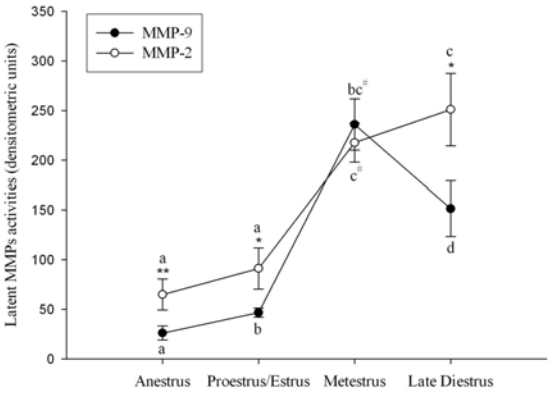

Figure 2 The activities of the latent form of endometrial MMPs in different phases of the estrous cycle. The superscripts indicate significant differences between different phases of the estrous cycle; *, indicates a significant difference between MMP-2 and MMP-9 in the same phase (*, $p<0.05$; **, $p<0.01$); #, indicates the superscript "bc" represent the MMP-2 group; superscript "c" represents the MMP-9 group.

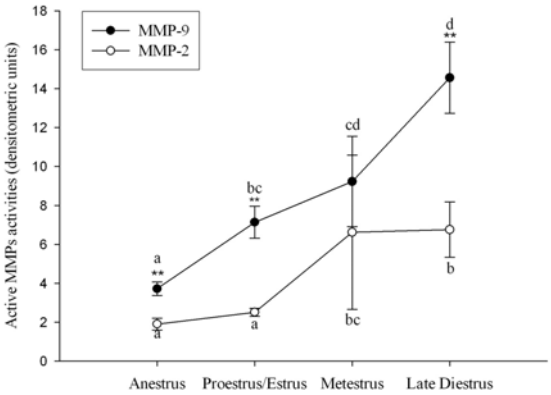

Figure 3 The activities of the active forms of endometrial MMPs in different phases of the estrous cycle. The superscripts indicate significant differences between different phases of the estrous cycle; **, indicates a significant difference between MMP-2 and MMP-9 in the same phase ($p<0.01$).

and active forms of MMP-2 were increased in MP (217.5±19.7 and 6.6±4.0, respectively) and reached the highest value in L-DP (250.9±36.6 and 6.6±4.0, respectively). The activity in L-DP was significantly higher than that in AP (64.9±15.6 and 1.9±0.3, respectively) and EP (91.3±20.7 and 2.5±0.2, respectively) ($p<0.01$) (Fig 2 and 3). The activities of latent MMP-2 were significantly higher than latent MMP-9 ($p<0.05$), but the activities of active MMP-9 were significantly higher than active MMP-2 ($p<0.01$) during the estrous cycle except in the state of MP (Fig 2 and 3).

The estradiol and progesterone concentrations in different phases of the estrous cycle are shown in table 2. During the estrous cycle, estradiol tended to negatively correlate with both latent and active forms of MMPs but without a significant difference. Progesterone tended to positively correlate with both active and latent forms of MMPs, especially in latent MMP-9 ($r= 0.3$, $p<0.05$) (Fig 4). The correlation coefficients of sex hormones and MMPs in different phases of the estrous cycle are presented in table 3. The correlations between plasma estradiol concentrations and the activities of MMPs were all without significant differences in any phase of the estrous cycle. Plasma progesterone positively correlated with active MMP-9 in MP ($r= 0.94$, $p<0.01$), and negatively correlated with latent MMP-9 in MP ($r= -0.83$, $p<0.05$) (Fig 5).

Discussion

The associations between plasma sex hormones and the activities of endometrial MMP-2 and -9 during the canine estrous cycle were revealed in this study. Activities of MMPs were increased in the phases after ovulation, which resulted in the formation of the corpus luteum and elevated progesterone concentrations. Thus, the effect of progesterone on the level of activities of MMPs was obviously displayed during MP, the phase with the highest plasma progesterone concentration. The timing of progesterone's effects on the activity and changes in endometrial MMPs in bitches was coincident with that in humans. However the expression of almost all MMPs, except MMP-2, was inhibited in contrast to the elevated endometrial MMP activities in bitches in our study (Marbaix et al., 1992; Rodgers et al., 1993; Rodgers et al., 1994; Salamonsen et al., 1997). The differences of endometrial MMP expression may be due to the fact that primate endometrium undergoes dramatic tissue sloughing and remodeling during the menstrual cycle, while canine endometrium remodeling occurs during late diestrus and is marked by widespread endometrial apoptosis, flattening of basal glandular epithelium, and loss of stromal collagen (Rehm et al., 2007).

Table 2 Sex hormones in different phases of the estrous cycle

Sex Hormones	Estrous Cycle			
	Anestrus	Proestrus/Estrus	Metestrus	Late Diestrus
Estradiol (µg/ml)	14.3±2.0	42.9±6.2	41.3±22.4	26.5±4.8
Progesterone (ng/ml)	0.6±0.0	3.0±0.8	55.4±12.1	1.2±0.2

All data are presented as mean±SEM

Table 3 Correlation coefficients of sex hormones and MMPs

Estrus Cycle	Correlation Coefficient		*P* value	
	Estradiol	Progesterone	Estradiol	Progesterone
Whole Estrus Cycle				
Latent MMP-9	0.00	0.32	0.97	0.01*
Active MMP-9	0.05	0.06	0.69	0.62
Latent MMP-2	-0.06	0.03	0.62	0.79
Active MMP-2	-0.05	0.07	0.60	0.56
Anestrus				
Latent MMP-9	0.08	0.48	0.80	0.11
Active MMP-9	-0.34	0.64	0.28	0.58
Latent MMP-2	0.03	0.00	0.93	1.00
Active MMP-2	-0.36	0.32	0.25	0.31
Estrus				
Latent MMP-9	-0.17	-0.04	0.39	0.85
Active MMP-9	-0.34	-0.14	0.09	0.51
Latent MMP-2	0.03	-0.35	0.90	0.08
Active MMP-2	-0.22	-0.10	0.28	0.62
Metestrus				
Latent MMP-9	-0.03	-0.83	0.96	0.04*
Active MMP-9	-0.26	0.94	0.62	< 0.01*
Latent MMP-2	-0.77	0.09	0.07	0.87
Active MMP-2	-0.14	0.60	0.79	0.21
Late Diestrus				
Latent MMP-9	0.09	0.19	0.71	0.43
Active MMP-9	0.10	-0.09	0.67	0.70
Latent MMP-2	-0.16	0.24	0.50	0.30
Active MMP-2	-0.23	-0.02	0.33	0.94

*significant correlation

Parallel increases in both forms of MMP-2 and -9 were observed in phases after ovulation. Angiogenesis, myometrial smooth muscle cell proliferation, and luminal epithelium degeneration are seen from EP to DP (Chu et al., 2006; Rehm et al., 2007). The elevation of MMPs during the metestrus phase in this study, may be due to the involvement of MMPs in angiogenesis (Rundhaug, 2005). During this period, enlarged blood vessels, erythrocyte extravasation, and blood flow into the uterine lumen are seen (Groppetti et al., 2010). In contrast to Chu and colleagues' study (Chu Py et al., 2002), significantly increased MMPs were found during L-DP in our study. Even though the duration of DP greatly varies with a range of 2-3 months (Rehm et al., 2007), luminal epithelium degeneration occupies a small part of DP. The involvement of MMPs in endometrial luminal epithelium degeneration still needs further studies. In human studies, MMP enzymes are secreted as latent forms and activated extracellularly by a variety of enzymes, including other MMPs (particularly MMP-3, MMP-9 and MT1-MMP) and by leucocyte proteases such as tryptase and elastase (Salamonsen and Lathbury, 2000). In our study, significantly increased activity of the active form of MMP-9 may result from the infiltration of neutrophils, lymphocytes, and macrophages and subsequent activation of latent MMP-9 during MP (Groppetti et al., 2010). In addition, increased leucocytes may contribute to the elevation in the level of MMPs (Vu et al., 1998; Yu et al., 1998). Although progesterone is a prominent sex hormone which may cause an increase of endometrial leucocytes during MP, researchers have revealed that neutrophils and macrophages do not express PR (or ERα) and hence regulation must be indirect (Stewart et al., 1998).

The phases of elevated endometrial MMP activities in our study coincided with the period of endometrial morphology changes and receptivity of the blastocyst. In this period, loss of microvilli from endometrial epithelial cells and development of membrane projections for successful implantation have been displayed in rats and humans (Psychoyos and Mandon, 1971; Enders and Nelson, 1973; Nikas et al., 2000; Nardo et al., 2002). Patients with unexplained infertility and implantation failure after *in vitro* fertilization have significantly lower MMP-2 (Konac et al., 2009). Although the expression of MMP-2 and MMP-9 mRNA did not change after the decidual treatment of rat endometrial stromal cells, the active forms of MMP-2 and MMP-9 significantly increased after *in vitro* decidualization (Matsumoto et al., 2009). During embryonic development, endometrial remodelling requires the cooperative actions of numerous MMPs which are involved in multiple processes required for reproductive success (Curry and Osteen, 2001).

Conclusion

In conclusion, the study provided useful information on the endometrial activities of latent and active forms of MMP-2 and MMP-9 during different phases of the canine estrous cycle. Latent forms of both MMP-2 and MMP-9 were the predominant forms. The activities of both forms of MMP-2 and MMP-9 were increased and accompanied by increased progesterone concentrations. This study revealed the association between endometrial MMP-2/MMP-9 activity and sex hormones and suggested that progesterone directly or indirectly regulated the MMP-2 and MMP-9 activity. However, the specific mechanism still needs further investigation.

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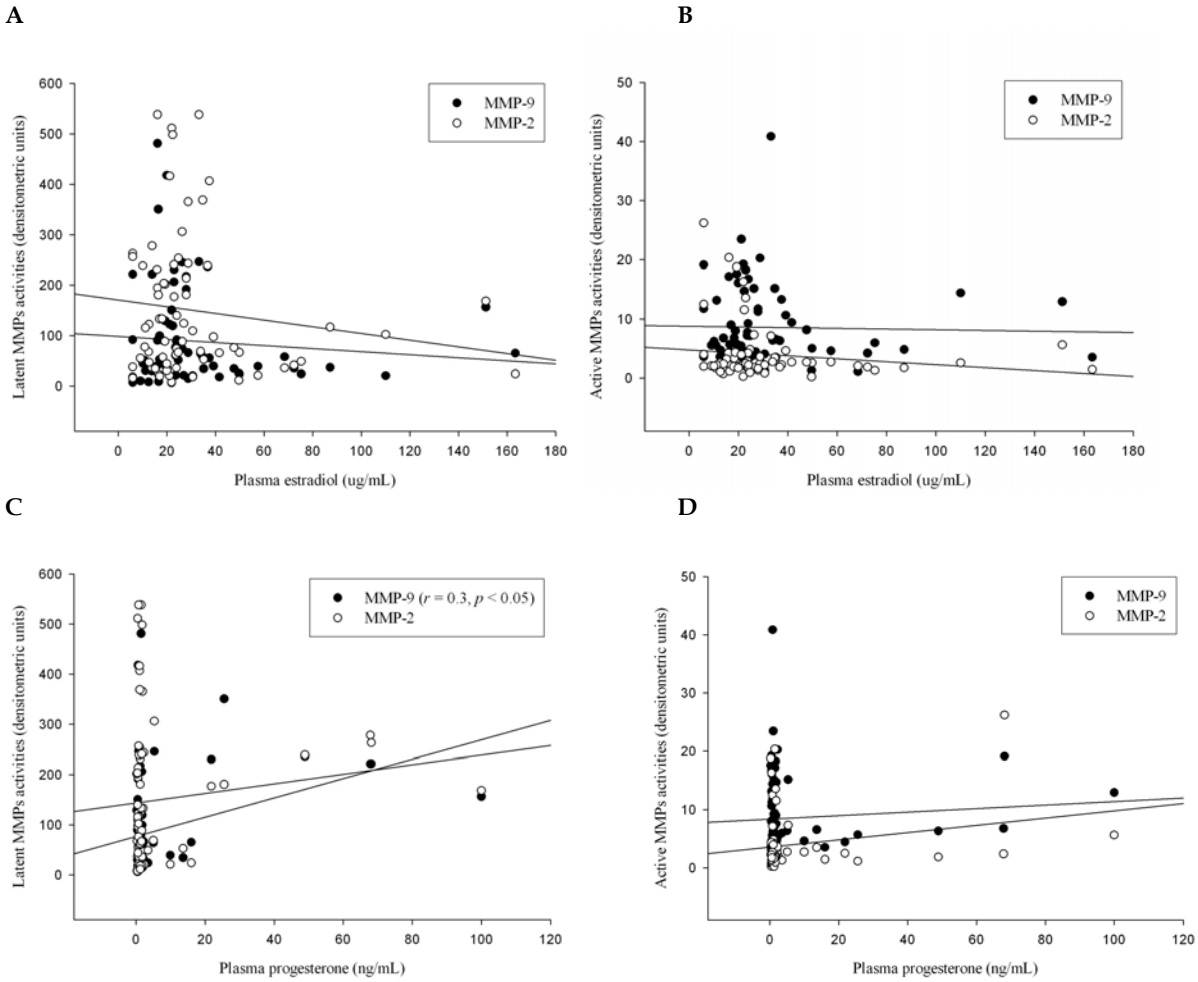

Figure 4 The correlation between sex hormones and activities of latent and active forms of endometrial MMPs. The correlation coefficient and p value will show in the results that have a significant correlation. A: The correlation between plasma estradiol and latent forms of MMPs; B: The correlation between plasma estradiol and active forms of MMPs; C: The correlation between plasma progesterone and latent forms of MMPs. Significantly positive correlations were found between progesterone and the activity of the latent form of MMP-9 (r= 0.3, p<0.05); D: The correlation between plasma progesterone and active forms of MMPs.

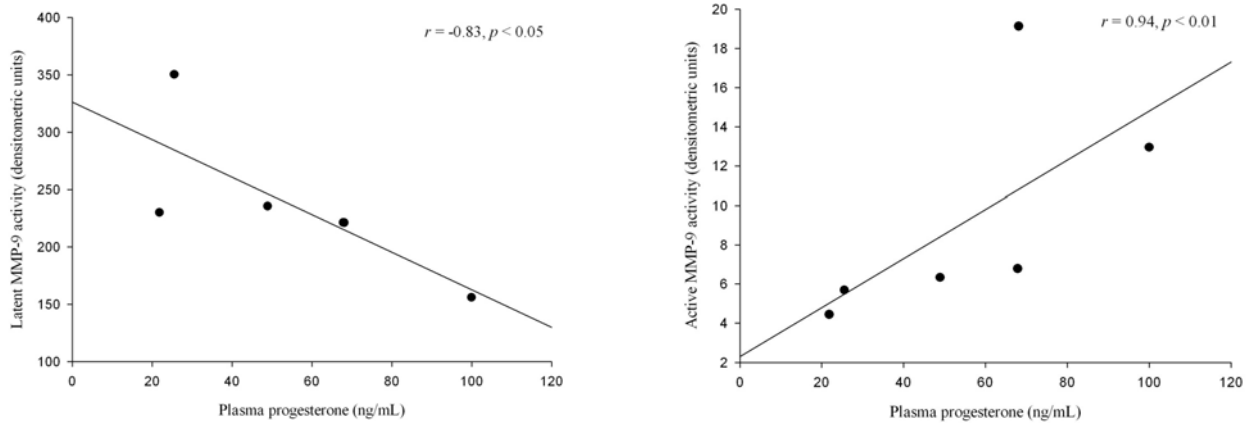

Figure 5 The correlation between plasma progesterone and the activity of endometrial MMP-9 in the metestrus phase. A: The correlation between progesterone and activity of the latent form of MMP-9. A significantly negative correlation was found between progesterone and the activity of the latent form of MMP-9 (r= -0.83, p<0.05); B: The correlation between progesterone and activity of the active form of MMP-9. A significantly positive correlation was found between progesterone and activity of the active form of MMP-9 (r= 0.94, p<0.01).

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