Theriogenology xxx (2014) 1–4

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Coagulation parameters do not change during luteal phase and pregnancy in cats

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ARTICLE INFO

Article history:
Received 19 November 2013
Received in revised form 18 March 2014
Accepted 20 March 2014

Keywords:
Queen
Pregnancy
Progesterone
Coagulation

ABSTRACT

Changes in coagulation parameters depending on reproductive status and pregnancy have been previously reported in both human and other veterinary species. The objective of this study was to determine if different reproductive status affects coagulation parameters in queens. Blood samples from 66 queens submitted to spay surgery were obtained. A hemostatic panel including platelet count, prothrombin time, activated partial thromboplastin time, fibrinogen concentration, and D-dimer and also progesterone concentrations were measured before surgery. According to progesterone results and embryo vesicles diameter, four groups were established: (1) nonpregnant queens with low (≤1 ng/mL) progesterone concentration (LP) (n = 33); (2) nonpregnant queens with high (≥2 ng/mL) progesterone concentration (n = 8) (HP); (3) first half of pregnancy (n = 12); and (4) second half of pregnancy (n = 13). None of the evaluated parameters showed statistically significant differences among the different groups. There was no significant linear correlation between progesterone values and coagulation parameters. In conclusion, neither the presence of the embryo nor the higher values of progesterone concentration induced statistically significant changes in the coagulation profile studied.

1. Introduction

Changes in the hemostatic system, often referred to as a hypercoagulable state with an increased activation of blood coagulation and fibrinolytic system, have been previously described in pregnant women [1,2]. This is associated with a higher risk of deep venous thromboembolism and obstetric disseminated intravascular coagulation [2]. The main changes include increases in the levels of clotting factors, decrease in anticoagulants, and reduction of fibrinolytic activity presumably to decrease the possibility of hemorrhage during pregnancy and delivery [2–4]. Some studies have related these modifications of the coagulation system to hormonal changes, particularly with female sex steroids [5,6].

Changes in coagulation have also been previously described in pregnant bitches. The main changes are hyperfibrinogenaemia [7–10] and increased concentration of fibrinogen degradation products [9]. On the contrary to what have been observed in women, hemostatic changes are not related to hormonal levels in bitches, but apparently to the inflammation induced by the presence of the embryo in the uterus [8,10].

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0093-691X/$ – see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.theriogenology.2014.03.022

Hemostatic status is routinely studied by platelet count, prothrombin time (PT), activated partial thromboplastin time (aPTT), and fibrinogen concentration. In addition to these tests, D-dimer evaluation has been recently included to evaluate coagulation status in both human [11,12] and veterinary medicine [13,14]. D-dimers, specific degradation products of cross-linked fibrin, are markers of activation of plasma coagulation and/or fibrinolysis [15]. D-dimer antigens are released during the breakdown of cross-linked fibrin, and their presence in plasma indirectly implies activity of thrombin, clotting factor XIII, plasmin, and thus formation of insoluble fibrin in the vascular system [16]. D-dimer concentration increases in coagulation disturbances, and this parameter is used to diagnose several pathologic states including early detection of gestational problems in women [17]. In dogs, increased concentrations of D-dimer were detected in patients with thromboembolic events, hepatopathy, neoplastic, cardiac, or renal disorders, after surgical intervention, and in animals with hemorrhage. Moreover, D-dimer concentration was also increased in dogs suffering from disseminated intravascular coagulation [18–21]. In contrast to the studies in human beings and dogs, few studies are available on D-dimer utility in cats for detection of hemostatic abnormalities in patients with liver disease and cardiomyopathy with variable results [22–24]. Establishment of the role of D-dimer concentration in human pregnancy is hampered because of the substantial increasing of concentration of D-dimer throughout gestational age [25,26].

The aim of the present study was to determine if pregnancy induces changes in the coagulation status and if these changes are related to high progesterone levels or to the presence of the embryo in queens.

2. Material and methods

2.1. Animals and sample collection

A total of 66 queens were included in the present study. All the females were included in a neutering program for stray animals. Previously to the surgery, a physical examination was performed and feline leukemia virus and feline immunodeficiency virus test (Snap FIV/FeLV; Idexx laboratories, Westbrook, ME, USA). Queens positive to any of these two diseases were excluded from the study.

Queens were premedicated with a combination of 5 mg/kg ketamine, 20 μg/kg buprenorphine, and 0.2 mg/kg midazolam intramuscularly. Then, a blood sample from the jugular vein was collected into glass and citrate tubes. Blood from glass tubes was allowed to clot and then centrifuged at 2200× *g* for 10 minutes for serum obtention. Citrate tubes were also centrifuged using the same method, and citrated plasma was collected. Serum and plasma were kept frozen at −20 °C until analyses were performed. Serum was used to determine progesterone concentration, whereas plasma was used to establish coagulation status.

After blood collection, the queens were induced with 2 to 4 mg/kg propofol intravenously and maintained with 1.5% to 2% isoflurane in oxygen through a Mapleson F anesthetic breathing circuit. Ovariohysterectomy surgery was performed by conventional ventral midline laparotomy. Immediately after removing the reproductive tract, uterus was incised and the diameter of embryo vesicles, if present, was measured to establish the gestational age [27].

According to progesterone results and embryo vesicles' diameter, four groups were established: (1) nonpregnant queens with low (≤1 ng/mL) progesterone concentration (LP) (n = 33), (2) nonpregnant queens with high (≥2 ng/mL) progesterone concentration (n = 8) (HP), (3) first half of pregnancy (n = 12), and (4) second half of pregnancy (n = 13).

2.2. Progesterone concentration

Progesterone concentration was determined from serum samples with an Immulite 1000 equipment (Immulite; Siemens Healthcare Diagnostics, Cornellà del Llobregat, Barcelona, Spain). Progesterone value was used to establish if ovulation had occurred or not in the nonpregnant queens. Those queens whose progesterone concentration was 1 ng/mL or less were included in the not-ovulated group, whereas those with progesterone concentration was 2 ng/mL or greater were included in the ovulated group.

2.3. Coagulation status

Coagulation panel included fibrinogen concentration, aPTT, PT, and D-dimer concentration. Activated partial thromboplastin time (C.K. Prest for determination of the Kaolin-aPTT; Diagnostica Stago S.A.S, Asnieres, France), PT (neoplastine CI plus for determination of PT; Diagnostica Stago S.A.S), and fibrinogen concentration (Fibri-prest 2 for quantitative determination of fibrinogen according to Class; Diagnostica Stago S.A.S) determinations were carried out on a single semi-automated coagulometer (Start4 hemostasis analyser; Diagnostica Stago S.A.S) according to manufacturer recommendations. D-dimer concentration was analyzed using an Olympus AU400 equipment (Olympus, Barcelona, Spain) with D-dimer Tinaquant Unisys immunoturbidimetric assay (Roche Diagnostics S.L., Madrid, Spain). D-dimer calibrator Gen and control I/II D-dimer Gen (Roche Diagnostics S.L.) was used as control material for D-dimer assay.

2.4. Statistical analysis

The commercial software SPSS for Windows, version 19 (SPSS, Chicago, IL, USA) was used for all statistical analyses. Kolmogorov–Smirnov test and the Shapiro–Wilk test were used to assess normal distribution of the parameters. The parameters showed a non-normal distribution and were analyzed through a Mann–Whitney nonparametric test. All values are expressed as median (percentile 2.25–97.75). Correlation among the evaluated coagulation parameters was assessed by applying a Spearman correlation test. Significance was set at $P < 0.05$ for all tests.

Two different approaches were applied. First, coagulation status was compared between low (≤1 ng/mL) and high (≥2 ng/mL) progesterone concentrations regardless of gestational age. Second, coagulation status was compared among the four different groups, taking into account the different gestational stages. Type I error correction for