



Blood loss and coagulation profile in pregnant and non-pregnant queens undergoing elective ovariohysterectomy

Journal of Feline Medicine and Surgery
2021, Vol. 23(6) 487–497
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DOI: 10.1177/1098612X20959610
journals.sagepub.com/home/jfm

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Abstract

Objectives The aims of this study were to determine if there is increased risk of intraoperative bleeding in pregnant cats undergoing elective ovariohysterectomy (OHE), and to compare coagulation in queens in various stages of estrus and pregnancy subjected to elective OHE using a whole-blood viscoelastic assay.

Methods Intraoperative blood loss was compared between non-pregnant and pregnant cats undergoing elective OHE. Viscoelastic evaluations of whole blood drawn pre- and postoperatively were performed using a point-of-care device measuring clot time (CT), clot formation time (CFT), alpha angle, maximum clot formation (MCF), amplitude at 10 and 20 mins (A10 and A20, respectively), and lysis index at 30 and 45 mins after MCF (LI30 and LI45, respectively).

Results One hundred and ninety-three cats underwent OHE by a ventral midline approach. Median blood loss was greater for pregnant cats (2.0ml, range <0.5–13ml) than non-pregnant cats (<0.5ml, range <0.5–15ml; $P < 0.0001$). Preoperatively, pregnant cats had a shorter median CFT (165s vs 190.5s), increased median A10 (31 from 25.5 VCM units) and A20 (38 from 35 VCM units), and a lower median LI45 (99% from 100%) than non-pregnant cats. Postoperatively, A10 and A20 increased, and LI30 and LI45 decreased in both non-pregnant and pregnant queens. In pregnant queens, mean CT also increased postoperatively.

Conclusions and relevance Pregnant cats were relatively hypercoagulable and had an increased rate of clot lysis than non-pregnant cats. Intraoperative blood loss was increased in pregnant vs non-pregnant cats, but no clinically relevant bleeding conditions occurred.

Keywords: Pregnancy; ovariohysterectomy; veterinary; viscoelastometry; coagulation; hemostasis; estrous cycle

Accepted: 24 August 2020

Introduction

In cats, reported complication rates associated with elective ovariohysterectomy (OHE) vary from 2.6% to as high as 20%, including rare fatal postoperative hemorrhage.^{1,2} In a study of 113,906 dogs and cats undergoing elective spay and neuter surgeries in a high-volume clinic, cats had higher perioperative mortality than dogs, and deaths were more common for female than male cats.³ Of the cats that died, 18% were pregnant, suggesting that there is a need to investigate whether pregnancy is a risk factor for adverse events, such as potentially fatal hemorrhage, during OHE.

Changes in coagulation parameters during the reproductive cycle and pregnancy have been previously

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reported in humans and veterinary species.^{4,5} In humans, pregnant women are hypercoagulable, with increased activity of clotting factors, decreased anticoagulants and reduced fibrinolytic activity.^{4,6-9} These hemostatic changes contribute to a higher risk of deep vein thromboembolism and disseminated intravascular coagulation.⁶ Some studies suggest this hypercoagulable state is related to hormonal changes that protect against premature placental separation and secure hemostasis at parturition.^{10,11} Changes in coagulation have also been described in pregnant bitches, including hyperfibrinogenemia,^{5,12-14} higher platelet counts,¹⁵ faster clot formation¹⁵ and increased concentration of fibrinogen degradation products.¹³ Contrary to what has been observed in women, hemostatic changes do not appear to be related to hormonal levels in bitches. Rather, the hemostatic changes appear to be associated with the inflammation that an embryo incites in the uterus.^{12,14,15}

A previous investigation of 66 pregnant queens did not detect an association between either the presence of an embryo or higher progesterone concentrations and platelet count, prothrombin time, activated partial thromboplastin time (aPTT), fibrinogen concentration and D-dimer.¹⁶ However, testing of individual components of the hemostatic system may not provide a reliable assessment of the overall coagulation status of a patient. Viscoelastic testing measures the elastic shear modulus of whole blood as it clots and thus provides a more global assessment of clot formation and lysis, which may provide additional insights into the coagulation status of pregnant queens. This possibility is supported by a canine study that identified associations between both pregnancy and estrous cycle stages and viscoelastic parameters, but not prothrombin time or D-dimer concentrations.¹⁵

Viscoelastic testing has traditionally relied on cost-prohibitive and complex instruments,¹⁷ but a small point-of-care device has recently become available (VCM Vet; Entegriion). This device requires loading non-anticoagulated whole blood into a prewarmed single-use cartridge, which avoids the 30-min rest time recommended for analysis of citrated blood by thromboelastography (TEG) or rotational thromboelastometry (ROTEM).¹⁸ After blood is loaded into the cartridge, it flows into a narrow space between two glass slides, which induce platelet and contact pathway activation. The clot builds strength as more platelets become activated and amplification of coagulation factor activation generates increasing amounts of fibrin, which polymerizes and is crosslinked by factor XIII.¹⁹ Over time, fibrinolysis by plasmin and platelet mediated clot retraction become the dominant processes, resulting in clot breakdown.^{17,19} Using a proprietary process, the instrument monitors changes in the mechanical properties of the clot and generates a clot curve (Figure 1) and several parameters that reflect clot formation and lysis (Table 1).

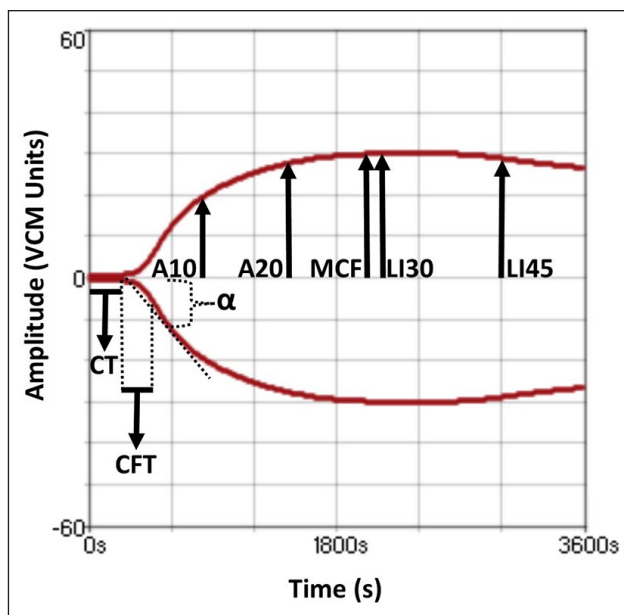


Figure 1 Typical VCM Vet (Entegriion) test clot curve tracing showing the commonly measured viscoelastic parameters: clot time (s) (CT), clot formation time (s) (CFT), alpha angle (α), maximum clot formation (VCM units) (MCF), amplitude (VCM units) at 10 and 20mins after CT (A10 and A20, respectively), and lysis index (%) at 30 and 45mins after CT (LI30 and LI45, respectively)

This study's primary objective was to determine if intraoperative blood loss is increased in pregnant vs non-pregnant cats undergoing elective OHE. A secondary objective was to compare viscoelastic coagulation parameters between pregnant and non-pregnant queens.

Materials and methods

This trial was a prospective, longitudinal, observational cohort clinical study. This study was approved by the Board of Directors of the Hill Country Animal League acting as an ad hoc institutional ethical review committee. Owner/agent consent was obtained before entering patients into the study.

Animals

The cohort included a combination of owned and feral/stray queens presented for elective OHE. Cats of ≥ 2.7 kg body weight and ≥ 4 months of age, as reported by the owner/agent and supported by the presence of permanent third incisors, were sequentially entered into the study. Included cats had no observed significant abnormalities noted on preoperative physical examinations that would have precluded their enrollment in the study. Cats were assigned to one of six subgroups intraoperatively, as determined by the surgeon: anestrus, estrus, postpartum, early pregnant, mid-pregnant or late pregnant. Anestrus was assessed when the queen's

Table 1 Summary of the parameters reported by the VCM Vet (Entegriion)

	Unit	Definition	Suggested corresponding coagulation process	Interpretation
CT	Seconds	Time from start of test to an increase in amplitude of 1% above baseline	Rate of coagulation factor activation leading to initial fibrin generation	Increased: hypocoagulable Decreased: hypercoagulable
CFT	Seconds	Time for an increase in amplitude from 1% to 10% above baseline	Rate of clot propagation, including coagulation factor amplification, fibrin polymerization and cross-linking, and platelet activation	
Alpha angle (α)	Degrees	The angle between the time axis and the tangent through the clot curve at 1% amplitude	Kinetics of clot formation	Increased: hypercoagulable Decreased: hypocoagulable
Amplitude (A10, A20)	VCM units	Amplitude, which is a measure of clot firmness, at 10 or 20 mins after CT	Measures of clot strength, which reflects contributions from multiple clot components, including fibrinogen, platelets, factor XIII and erythrocytes	Increased: hypercoagulable Decreased: hypocoagulable
MCF	VCM units	Maximum amplitude of the clot curve		
Lysis index (LI30, LI45)	%	Amplitude at 30 or 45mins after CT, as a percentage of MCF	Fibrinolysis and clot contraction	Decreased: faster clot breakdown/retraction

The suggested correspondence between the VCM Vet parameters and stages of coagulation are extrapolated from thromboelastography (TEG) and rotational thromboelastometry (ROTEM)¹⁷

CT = clot time; CFT = clot formation time; MCF = maximum clot formation

ovaries appeared quiescent. Estrus was assessed if the uterus was enlarged and active follicle formation was observed. Postpartum cats had mildly enlarged, flaccid uteri with inactive follicles. Early pregnant cats had enlarged, turgid, often tortuous uteri with evidence of ruptured follicles and/or corpus luteum development. Mid-pregnant queens had enlarged uteri with discrete fetal boli ('beads on a string') present. Queens considered to be in late pregnancy had notably enlarged uteri with large, coalescing fetuses assessed to be near term.

Anesthesia, analgesia and OHE

A standardized protocol for anesthesia and the recovery procedure was implemented by experienced veterinary technicians. Equal volumes of ketamine (100 mg/ml; Ketamine HCl [VEDCO]) and dexmedetomidine (0.5 mg/ml; Dexmedetomidine HCl [Putney]) were mixed in a single sterile vial. Anesthesia was induced by intramuscular injection of 0.4 ml of this mixture. The total dosage of induction drugs ranged from 4.2 mg/kg to 7.3 mg/kg for ketamine and from 0.02 mg/kg to 0.04 mg/kg for dexmedetomidine. Buprenorphine (0.01 mg/kg IM; Buprenorphine Injection PF [Roadrunner Pharmacy]) was administered after anesthetic induction, during surgical preparation. Each animal was intubated,

and isoflurane was administered to assist in maintaining a surgical plane of anesthesia with a vaporizer setting range of 0.5–1% (Isoflurane; MWI). Oxygen flow was 2 l/min using a non-rebreathing circuit. Anesthesia was monitored by the operating room technicians with oversight from the surgeon.

OHEs were performed by a standard midline celiotomy by one of three surgeons (>5 years of experience and >10,000 feline OHEs each in high-volume, high-quality feline surgical practice). All procedures were performed aseptically following published guidelines for elective surgery on shelter animals.²⁰ Surgical times were measured from initial incision to closure of the skin. Surgical anesthesia recovery was performed by solely dedicated, experienced veterinary technicians who remained with patients in a dedicated area until the end of the anesthetic period as determined by extubation, regaining consciousness and maintaining normal vital signs.

A primary outcome measure was the presence of increased intraoperative blood loss. A previously reported gravimetric method was used to determine blood loss.²⁰ Dry, sterile, 4" × 4" cotton surgical gauze sponges were weighed on a previously zeroed electronic scale before and after attempting to saturate the swab with free blood

within the peritoneal cavity. The difference in their weight was used to estimate blood loss, based on the assumption that the density of blood was 1 g/ml.^{20–24} If, on entering the abdominal cavity, peritoneal effusion fluid was identified, this was absorbed using a separate surgical sponge to avoid inclusion in the measured blood loss.

Coagulation status

Blood samples were drawn by a routine venipuncture from a medial saphenous vein using a 22 G needle preoperatively during surgical preparation, and 10 mins postoperatively or at the end of anesthesia recovery, whichever came first. Care was taken to reduce the tissue trauma associated with venipuncture by entering the vein on the first attempt. If entry into the vein on the first attempt was not successful, the sample was discarded, and the cat was not included in the study. All animals were anesthetized at the time of sample collection. Packed cell volumes (PCVs) and serum total protein (TP) via refractometry (Professional ATC Clinical Refractometer; National Industrial Supply) were measured from collected samples. Non-anticoagulated whole blood samples were analyzed immediately upon collection using a cartridge-based point-of-care viscoelastic instrument. This instrument uses a contact pathway activator; the manufacturer's protocol does not require blood to be rested before analysis (VCM Vet; Entegriion). The following viscoelastic parameters were compared to determine coagulation differences between pregnant and non-pregnant cats: clot time (s) (CT), clot formation time (s) (CFT), alpha angle (°) (α), maximum clot formation (VCM units) (MCF), amplitude (VCM units) at 10 and 20 mins after CT (A10 and A20, respectively), and lysis index (%) at 30 and 45 mins after CT (LI30 and LI45, respectively).

Statistical analysis

Pre- and postoperative PCV and TP were compared between groups using ANOVA. Multivariate linear regression analyses were performed to determine the relationship between PCV and TP, and viscoelastic coagulation parameters. CT, CFT, α , MCF, A10, A20, LI30 and LI45 were compared between pregnant and non-pregnant animals before and after surgery by *t*-test or Mann–Whitney U test, depending upon data distribution, as assessed by the D'Agostino–Pearson test. Pre- and postoperative viscoelastic parameters were compared by Wilcoxon test or a paired *t*-test. Comparisons of viscoelastic parameters between queens in early, mid- and late pregnancy, and between non-pregnant queens in estrus, anestrus and postpartum were performed by Kruskal–Wallis test, and, if significant, this was followed by Conover's test to identify statistically significant differences between individual groups. Statistical significance was set at $P < 0.05$.

Statistical analyses were performed using MedCalc Statistical Software version 19.1.3 (MedCalc Software; <https://www.medcalc.org>) and Excel 2016 (Microsoft).

Results

Cats

Two hundred thirty-six cats were entered into the study. Forty-three were excluded owing to a technical error in running samples, clerical errors, incomplete data due to an unexpected early recovery from anesthesia or medical/surgical complications. For the remaining 193 cats, 125 were non-pregnant (58 anestrus, 31 estrus, 36 postpartum) and 68 were pregnant (15 early, 21 mid and 32 late). All groups were similar with regard to breed. There were no significant differences among the subgroups regarding age (mean age was 16.9 ± 1.0 months). The late pregnant subgroup of cats was significantly heavier than anestrus cats, postpartum cats and those in early pregnancy, with an average body weight of 3.70 ± 0.52 kg vs 3.26 ± 0.43 kg, 3.36 ± 0.36 kg and 3.33 ± 0.34 kg, respectively ($P < 0.001$) (Table 2). Thirteen cats were reported by the owner/agent to be receiving preventive antiparasitic treatments (12/13 imidacloprid and moxidectin, 1/13 selamectin and sarolaner).

Surgical times

Surgical times were measured in 190/193 cats. Overall, the mean surgical time was 12 ± 4 mins (range 3–30 mins). Mean surgical times for late pregnant cats (15 ± 2 mins) were significantly greater than anestrus cats (10 ± 4 mins), estrus cats (11 ± 3 mins), postpartum cats (11 ± 2 mins) and early pregnant cats (11 ± 2 mins) ($P < 0.0001$). Mean surgical times for cats in mid-pregnancy (13 ± 4 mins) were significantly greater than anestrus cats (10 ± 4 mins) ($P < 0.01$).

Blood loss

The overall median blood loss was < 0.5 ml (range < 0.5 –15 ml). Pregnant cats (median 2.0 ml; range < 0.5 –13 ml) had greater blood loss than non-pregnant cats (median < 0.5 ml; range < 0.5 –15 ml) ($P < 0.0001$) (Table 3).

PCVs and serum proteins

PCV for late pregnant cats was lower than the other groups. The change in PCV values from preoperatively to postoperatively was not different between the groups. There were no significant differences in preoperative TP or change in TP between pre- and postoperative samples (Table 4). Regression analyses showed statistically significant relationships between PCV and CT, α , MCF, A10 and A20. Similarly, there were significant relationships for TP with CT, MCF, A10 and A20 (Table 5).

Table 2 Demographics for enrolled cats

Group	n	Breed (%)	Mean ± SD body weight (kg)	Mean ± SD age (months)
Non-pregnant	125	DSH (70) DMH (14) DLH (8) Siamese (7) Manx (1)	3.3 ± 0.40	17 ± 11.9
Anestrus	58	DSH (69) DMH (16) DLH (10) Siamese (5)	3.3 ± 0.43	15 ± 11.6
Estrus	31	DSH (68) DMH (16) DLH (10) Siamese (6)	3.4 ± 0.39	18 ± 9.7
Postpartum	36	DSH (72) DMH (11) DLH (3) Siamese (11) Manx (3)	3.4 ± 0.36	20 ± 13.6
Pregnant	68	DSH (68) DMH (21) DLH (7) Siamese (4)	3.6 ± 0.54	17 ± 8.7
Early pregnant	15	DSH (73) DMH (27) DLH (0) Siamese (0)	3.3 ± 0.34	14 ± 8.4
Mid-pregnant	21	DSH (67) DMH (24) DLH (5) Siamese (5)	3.5 ± 0.63	18 ± 9.0
Late pregnant	32	DSH (66) DMH (16) DLH (12) Siamese (6)	3.7 ± 0.52*	18 ± 8.5
Overall	193	DSH (69) DMH (17) DLH (8) Siamese (6) Manx (<1)	3.4 ± 0.46	17 ± 1.0

*Significantly ($P < 0.001$) higher mean body weight than anestrus, postpartum and early pregnant cats
DSH = domestic shorthair; DMH = domestic mediumhair; DLH = domestic longhair

Table 3 Summary of intraoperative blood loss (ml) in cats undergoing ovariohysterectomy

Groups	n	Median	Minimum	Maximum
Anestrus	58	0	0	15
Estrus	31	0	0	14
Postpartum	36	0	0	8
Early pregnant	14	0	0	8
Mid-pregnant*	21	2	0	10
Late pregnant†	32	0.5	0	13
Overall	192	0	0	15

*Mid-pregnant cats had greater intraoperative blood loss than anestrus ($P < 0.005$) and postpartum cats ($P < 0.01$)
†Late pregnant cats had greater intraoperative blood loss than anestrus cats ($P < 0.005$)

Table 4 Summary of packed cell volume (PCV) and serum total protein (TP) results for subgroups undergoing ovariohysterectomy

Group	Preoperative	Postoperative
PCV (%)		
Not pregnant		
Anestrus	42.3 ± 5.12	39.4 ± 3.51
Estrus	40.1 ± 7.20	36.1 ± 4.62
Postpartum	38.0 ± 6.25	34.1 ± 4.94
Pregnant		
Early	41.6 ± 7.37	37.9 ± 3.58
Mid	36.3 ± 4.38	32.2 ± 3.46†
Late	33.1 ± 4.82*	27.6 ± 2.89‡
TP (g/dl) via refractometry		
Not pregnant		
Anestrus	7.1 ± 0.23	7.0 ± 0.19
Estrus	7.3 ± 0.38	7.2 ± 0.51
Postpartum	7.2 ± 0.43	7.4 ± 0.84
Pregnant		
Early	6.8 ± 0.38	7.2 ± 0.71
Mid	7.3 ± 0.69	7.5 ± 0.60
Late	7.0 ± 0.49	6.7 ± 0.53§

Data are mean ± SD
*Preoperative late pregnant cats had a lower PCV than anestrus ($P < 0.001$) and estrus ($P < 0.005$) cats
†Postoperative mid-pregnant cats had a lower PCV than anestrus ($P < 0.001$) and early pregnant ($P < 0.01$) cats
‡Postoperative late pregnant cats had a lower PCV than anestrus ($P < 0.00005$), estrus ($P < 0.00001$), postpartum ($P < 0.005$), early pregnant ($P < 0.0001$) and mid-pregnant ($P < 0.001$) cats
§Postoperative late pregnant TP was lower than mid pregnant ($P < 0.001$)

Table 5 Regression analysis statistics for relationships of packed cell volume (PCV) and serum total protein (TP) to viscoelastic coagulation parameters from perioperative testing in cats undergoing ovariohysterectomy

	CT	CFT	α	MCF	A10	A20
R ²	0.05	-0.01	0.05	0.07	0.20	0.17
ANOVA <i>P</i> value	0.01	NS	0.01	<0.01	<0.001	<0.001
PCV <i>P</i> value	0.05	NS	<0.01	<0.01	<0.001	<0.001
TP <i>P</i> value	<0.05	NS	NS	0.04	<0.05	<0.05

CT = clot time; CFT = clot formation time; α = alpha angle; MCF = maximum clot formation; A10 and A20 = amplitude at 10mins and 20mins, respectively; NS = not significant

Table 6 Summary results of preoperative viscoelastic testing of cats undergoing ovariohysterectomy to compare non-pregnant with pregnant cats

	Non-pregnant median	Minimum	Maximum	Pregnant median	Minimum	Maximum	<i>P</i> value
CT (s)	325.5	29	724	340	36	1964	
CFT (s)*	190.5	74	3260	165	22	918	<0.01
α (°)	49.5	4	75	53	13	77	
A10*	25.5	3	40	31	6	54	<0.0005
A20*	35	4	50	38	13	60	<0.005
MCF	41	5	55	44	17	64	
LI30 (%)	100	83	100	100	10	100	
LI45 (%)*	100	57	100	99	81	100	<0.0001

*Statistically significant

CT = clot time; CFT = clot formation time; α = alpha angle; A10 and A20 = amplitude at 10 and 20 mins, respectively; MCF = maximum clot formation; LI30 and LI45 = lysis index at 30 and 45 mins, respectively

Table 7 Summary results of preoperative viscoelastic testing of cats undergoing ovariohysterectomy to compare non-pregnant cat subgroups: anestrus, estrus and postpartum

	Anestrus			Estrus			Postpartum			<i>P</i> value
	Median	Minimum	Maximum	Median	Minimum	Maximum	Median	Minimum	Maximum	
CT (s)	324.5	29	623	354	60	679	310	35	724	
CFT (s)	188	74	322	214*	104	3260	177	80	269	<0.01
α (°)	50	32	74	49	4	67	50	36	75	
A10	26	16	40	22*	3	38	28	18	40	<0.0001
A20	35	21	48	30*	4	47	37	26	50	<0.001
MCF	42	24	54	36*	5	53	43	33	55	<0.0005
LI30 (%)	100	98	100	100	83	100	100	98	100	
LI45 (%)	100	91	100	100	57	100	100	91	100	

*Statistically significant

CT = clot time; CFT = clot formation time; α = alpha angle; A10 and A20 = amplitude at 10 and 20mins, respectively; MCF = maximum clot formation; LI30 and LI45 = lysis index at 30 and 45 mins, respectively

Viscoelastic test results

Comparison of pregnant with non-pregnant queens Compared with non-pregnant cats, preoperative samples from pregnant cats had a shorter median CFT ($P < 0.01$), increased median A10 ($P < 0.0005$) and A20 ($P < 0.005$), and a lower LI45 ($P < 0.0001$) (Table 6). Cats in estrus had a longer median CFT ($P < 0.01$) and decreased median MCF ($P < 0.0005$), A10 ($P < 0.0001$) and A20 ($P < 0.001$) than

anestrus or postpartum cats (Table 7). Cats in early pregnancy had lower median A10 ($P < 0.01$) and A20 ($P < 0.05$) than cats in mid- or late pregnancy. Cats in late pregnancy had lower LI45 ($P < 0.005$) values than cats in early or mid-pregnancy (Table 8).

Effects of surgery on coagulation A10 and A20 were increased and LI30 and LI45 decreased postoperatively in

Table 8 Summary results of preoperative viscoelastic testing of cats undergoing ovariohysterectomy to compare pregnant cat subgroups: early pregnant, mid-pregnant and late pregnant

	Early pregnant			Mid-pregnant			Late pregnant			P value
	Median	Minimum	Maximum	Median	Minimum	Maximum	Median	Minimum	Maximum	
CT (s)	342.5	36	475	321	61	1964	340	56	814	
CFT (s)	180	22	578	167	50	918	144	106	340	
α ($^{\circ}$)	49.5	29	67	51	13	77	54	33	63	
A10	24.5*	10	34	32	6	54	31	14	44	<0.01
A20	33.5*	16	43	41	13	60	39	19	53	<0.05
MCF	40	21	50	46	17	64	42	22	57	
LI30 (%)	100	98	100	100	100	100	100	10	100	
LI45 (%)	100	81	100	100	94	100	98*	85	100	<0.005

*Statistically significant

CT = clot time; CFT = clot formation time; α = alpha angle; A10 and A20 = amplitude at 10 and 20mins, respectively; MCF = maximum clot formation; LI30 and LI45 = lysis index at 30 and 45 mins, respectively

Table 9 Summary of perioperative viscoelastic testing of cats undergoing ovariohysterectomy to compare preoperative with postoperative results

	Preoperative			Postoperative			P value
	Median	Minimum	Maximum	Median	Minimum	Maximum	
Non-pregnant							
CT (s)	325.5	29	724	354	44	992	
CFT (s)	190.5	74	3260	184	48	522	
α ($^{\circ}$)	49.5	4	75	49	25	77	
A10*	25.5	3	40	27	11	49	0.0001
A20	35	4	50	Mean 35.8	11	SD 7.25	
MCF	41	5	55	Mean 41.0		SD 7.57	
LI30 (%)*	100	83	100	100	91	100	<0.0001
LI45 (%)*	100	57	100	100	48	100	<0.005
Pregnant							
CT (s)*	340	36	1964	380.5	44	1636	<0.05
CFT (s)	165	22	918	173	52	860	
α ($^{\circ}$)	53	13	77	50.5	14	76	
A10	31	6	54	31	6	48	
A20	38	13	60	38.5	15	58	
MCF	44	17	64	43	19	63	
LI30 (%)*	100	10	100	100	94	100	<0.05
LI45 (%)*	99	81	100	99	85	100	<0.05

Non-pregnant, postoperative data for amplitude at 20 mins (A20) and maximum clot formation (MCF) were normally distributed; mean and SD are reported

*Statistically significant

CT = clot time; CFT = clot formation time; α = alpha angle; A10 = amplitude at 10 mins; LI30 and LI45 = lysis index at 30 and 45 mins, respectively

both non-pregnant and pregnant queens. In pregnant queens, mean CT was also increased postoperatively (Table 9).

Discussion

Intraoperative blood loss was significantly higher in pregnant compared with non-pregnant cats. However,

blood loss in both groups was low, and there were no clinically significant bleeding complications. Estimated feline blood volume is 60 ml/kg,²⁵ and the median blood loss of 2.0 ml in pregnant cats therefore represents approximately 0.8% of the circulating blood volume of a 4 kg cat. In non-anemic human patients, intraoperative blood loss of <15% of blood volume is not expected to cause clinical signs, and, in otherwise healthy people,

an intraoperative blood loss $\geq 30\%$ of blood volume has been recommended as a red cell transfusion trigger.²⁶ As blood loss was well below these thresholds, the current study demonstrates that elective OHE can be safely performed in queens at all stages of pregnancy. However, as pregnant queens lost more blood than non-pregnant animals even when OHEs were performed by experienced surgeons, our findings suggest that particular attention should be paid to hemostasis in pregnant cats, and that veterinary students and inexperienced graduates may be well served with additional support when operating on pregnant cats.

Viscoelastic testing identified statistically significant differences between pregnant and non-pregnant animals but, in most cases, results were within the reference intervals supplied by the manufacturer. This supports the conclusion that increased blood loss in pregnant cats reflects increased tissue vascularity and greater surgical complexity, rather than an underlying hemostatic disturbance. While the viscoelastic changes are likely of little clinical relevance in this population of apparently healthy cats, they provide insights into the coagulation status of pregnancy, which are both physiologically interesting and may, in the future, be relevant to clinical management in more high-risk situations such as dystocia.

Viscoelastic testing identified that in pregnant cats, propagation of the fibrin clot was accelerated, as shown by the decreased CFT, resulting in a stronger clot during the early phases of clot formation, as measured by the increased A10 and A20. Pregnant queens also displayed accelerated clot breakdown and/or retraction, as shown by the decreased LI45. Neither thrombosis nor bleeding are commonly reported in pregnant cats,²⁷ suggesting that this combination of hypercoagulable and hyperfibrinolytic changes typically results in a relatively balanced homeostatic state. However, increased fibrinolysis during pregnancy is potentially interesting from the perspective of diagnosis and treatment of bleeding disorders in pregnant cats. The antifibrinolytic agent tranexamic acid reduces death in women with postpartum hemorrhage,²⁸ and for women undergoing cesarian section, its prophylactic use decreases perioperative blood loss and transfusion requirements.²⁹ It should be emphasized that there is currently a lack of information available regarding the safety or efficacy of antifibrinolytics in feline patients.³⁰

Using individual measures of hemostatic status, such as platelet concentrations and aPTT, Rivera del Álamo et al¹⁶ found no evidence of changes in coagulation parameters between pregnant cats and non-pregnant cats, or between non-pregnant cats at different stages of their reproductive cycles.¹⁶ This is contrary to our findings, where, in addition to the differences between pregnant and non-pregnant cats, there were statistically significant alterations in viscoelastic parameters between cats in estrus and anestrus or postpartum animals, and between

cats at different stages of pregnancy. Specifically, estrus was associated with hypocoagulable changes, including weaker clot strength and longer clot formation times, and the hypercoagulable and hyperfibrinolytic changes observed in pregnant cats were more marked in late-stage pregnancy. The differences between our findings and the previous study can likely be explained by the more global assessment of coagulation status provided by viscoelastic testing.¹⁷ It should be emphasized that while these findings are physiologically interesting, they are unlikely to be of immediate clinical relevance as no cats in the current study had spontaneous or surgery-associated bleeding or thrombotic disorders and in most cases, changes in viscoelastic parameters were relatively subtle.

In this study, cats in late pregnancy had lower PCVs than other groups. This is consistent with people, in whom hemoglobin concentration, hematocrit and red cell count fall during pregnancy because the expansion of the plasma volume is greater than that of the red cell mass.³¹ Reduced PCV is associated with increased clot strength in TEG or ROTEM, and it is suggested that this effect is largely an *in vitro* artifact, rather than necessarily an indication of clinically relevant hypercoagulability.³² Although the effect of PCV on the viscoelastic test device used for this study has not yet been investigated, our regression analysis does suggest a similar association between decreasing PCV and apparent hypercoagulability. However, the regression coefficients were relatively low, suggesting that the hypercoagulability in pregnant queens is unlikely to be explained by decreased PCV alone.

Surgery (OHE) also appeared to influence viscoelastic parameters. Postoperatively, the strength of clots formed increased (as shown by increased A10 and A20), but so did their rate of lysis (as shown by decreased LI30 and LI45). The increase in postoperative strength of clots formed is consistent with previous findings in dogs undergoing splenectomies and orthopedic surgery.^{33,34} Several factors can affect perioperative coagulation, including anesthesia. In the current study, samples were collected after induction of anesthesia. Sharma and Philip³⁵ showed that the use of general anesthesia for cesarean section in people is associated with accelerated coagulability when compared with spinal anesthesia. Griffin et al³⁶ showed that, in rats, buprenorphine analgesia had possible research implications. They demonstrated a significantly increased clot strength without affecting fibrinolysis, and increased plasma fibrinogen in rats anesthetized with isoflurane and buprenorphine analgesia. A previous study in cats found that ketamine may slightly increase prothrombin time and partial thromboplastin time after intravenous administration in combination with diazepam.³⁷ However, the observed changes were considered small and likely not of clinical relevance.³⁷ Chen et al³⁸ investigated the effect

of dexmedetomidine on blood coagulation in patients undergoing gastrectomy. In this study, although the postoperative coagulation system was activated, the inclusion of dexmedetomidine in the anesthesia protocol had a beneficial effect on postoperative coagulation.³⁸ Owing to the lack of studies using point-of-care viscoelastic testing in cats, the extent to which prior anesthesia may have influenced results in this study is unclear. To avoid the influence of the above factors on coagulation results, we limited our observations to one type of surgery and a commonly used standardized anesthesia protocol. However, we used a fixed dose of ketamine and dexmedetomidine regardless of body weight, and as late pregnant cats were heavier than other groups, this may have influenced our findings.

Our assessment of intraoperative blood loss relied on a simple gravimetric technique. It is possible that gravimetric quantification of blood loss, by weighing sponges, may underestimate true blood loss.²⁵ A previous veterinary study found gravimetric assessment to be an accurate technique for estimation of intraoperative blood loss but did report that evaporation could lead to underestimation of blood loss.²⁴ Given the short duration of the surgeries in the current study, we do not think evaporation was likely to have had a major impact on our estimation of blood loss. However, it is possible that other factors may have introduced inaccuracy, including variability between surgeons in their technique for saturating sponges; sequestration of blood within the ligatures; or blood lost and dried onto drapes, gloves and instruments. Additionally, some cats, in particular pregnant queens, may have a small amount of free fluid in the abdomen. While efforts were made to remove this fluid when entering the abdomen at the start of the procedure, if present, it is possible that some of this fluid could have been collected along with blood lost during surgery and thereby falsely increased the estimated intraoperative blood loss. It is also important to note that in most cases, blood loss was minimal, and for these animals, the amount of blood loss likely falls below the volume that could be accurately assessed by gravimetric techniques.

Other limitations included the relatively limited information available about the health status of cats in the current study. Patients consisted of a combination of owned and stray/feral cats presented to a high-volume surgical center for an elective procedure. Given the limited diagnostics and limited ability for collecting long-term medical history or follow-up, it is difficult to completely rule out significant underlying health conditions. Notably, feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV) status were not determined, and coagulation alterations, such as prolonged aPTT, have been reported in apparently healthy cats that are seropositive for FIV or FIV/FeLV.³⁹ Similarly, few of the cats were receiving

heartworm preventatives, and *Dirofilaria immitis* antigens have promoted fibrinolysis by accelerating conversion of plasminogen to plasmin.⁴⁰ As our data set could include cats with unrecognized underlying health conditions, our results should not be considered reference intervals for healthy cats at different stages of the reproductive cycle.

Although training and coordination were conducted prior to the study to limit bias and improve precision, the determination of estrous cycle and pregnancy status was a subjective clinical decision made at the discretion of the individual surgeon based upon the surgical anatomy of the individual patient. It is also important to note that a full validation study has yet to be published for analysis of feline samples by the viscoelastic instrument used in the current study. Determining analytical and inter- and intra-animal coefficients of variation for this instrument is therefore an important next step for interpreting the likely physiologic and clinical significance of the differences observed between pregnant and non-pregnant cats.

There is also a need for large-scale studies addressing the sensitivity and specificity of changes in the analytical parameters for prediction of bleeding or thrombosis, and the effect of pre-analytical variables, such as delayed cartridge filling or traumatic venipuncture. As pre-analytical and analytical factors could have affected our results, we feel it is important to emphasize that pregnant cats with bleeding disorders should continue to be thoroughly evaluated, rather than assumed to be affected by hyperfibrinolysis.

Conclusions

During elective OHE, pregnant cats had increased intraoperative blood loss vs non-pregnant animals. However, no serious bleeding complications occurred in either group. Viscoelastic testing identified that pregnant cats were relatively hypercoagulable with an increased rate of clot lysis compared with non-pregnant cats, but these changes were generally small and likely of limited clinical significance.

Acknowledgements The authors thank Faith Northcutt, Will Northcutt, Kaylie Kelsey and Devin Angelucci for their technical support, especially with postoperative recovery and sample collection. The authors also thank Katherine Cano for technical assistance with operating room procedures, data collection and laboratory support. Further, the authors extend their gratitude to Bernadette Vogel for study administrative support and Sandra Montalbo for table design.

Conflict of interest The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding This work was supported by the Duffield Family Foundation, Maddie's Fund (May 11, 2018).

Ethical approval This work involved the use of non-experimental animals only (including owned or unowned animals and data from prospective or retrospective studies). Established internationally recognized high standards ('best practice') of individual veterinary clinical patient care were followed. Ethical approval from a committee, while not necessarily required, was nonetheless obtained, as stated in the manuscript.

Informed consent Informed consent (either verbal or written) was obtained from the owner or legal custodian of all animal(s) described in this work (either experimental or non-experimental animals) for the procedure(s) undertaken (either prospective or retrospective studies). No animals or humans are identifiable within this publication, and therefore additional informed consent for publication was not required.

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