Influence of sex on activated partial thromboplastin time (aPTT) and prothrombin time (PT) in sheep

Maria Marília Leite Carlos[1], Marília Martins Melo[2] and Benito Soto-Blanco[2*]

[1] Department of Biomedical Sciences, Faculdade de Ciências da Saúde, Universidade do Estado do Rio Grande do Norte (UERN), Rua Miguel Antônio da Silva Neto, Mossoró, RN 59607-360, Brazil.
[2] Department of Veterinary Clinics and Surgery, Escola de Veterinária, Universidade Federal de Minas Gerais (UFMG), Avenida Antônio Carlos 6627, Belo Horizonte, MG 31270-901, Brazil.

[*] Corresponding author at: Department of Veterinary Clinics and Surgery, Escola de Veterinária, Universidade Federal de Minas Gerais (UFMG), Avenida Antônio Carlos 6627, Belo Horizonte, MG 31270-901, Brazil.
Tel.: +5531 3409 2255, e-mail: benito.blanco@pq.cnpq.br.

Veterinaria Italiana 2017, **53** (3), 255-258. doi: 10.12834/Vetlt.278.1021.3
Accepted: 22.04.2016 | Available on line: 30.08.2017

Keywords
Blood coagulation,
Clotting time,
Haemostasis,
Small ruminants.

Summary
Haemostasis is a physiological process that prevents excessive blood loss. In laboratory, the prothrombin time (PT) and the activated partial thromboplastin time (aPTT) are used to examine clotting systems. However, the influence of sex on PT and aPTT values is unknown. The present work aimed to determine the values for PT and aPTT in adult sheep and to evaluate their dependence on the sex of the animal. Blood samples were collected from 40 adults (1-6 years old) of mixed breed sheep (20 males and 20 females) via jugular venepuncture conducted using vacuum tubes containing 3.8% sodium citrate as an anticoagulant. PT and aPTT were determined by visual detection of clot formation. The mean PT and aPTT values for all sheep were 7.71 ± 0.87 s and 35.7 ± 3.57 s, respectively. The aPTT values showed a significant difference (P = 0.0013) between male and female samples, while the difference in PT values was not significant (P = 0.0565). Thus, the animal sex influences the function of the plasma blood-clotting system in sheep. In contrast with table 1 data, in particular, aPTT values are significantly higher in female sheep than in males.

Influenza del sesso sul tempo di tromboplastina parziale attivata (aPTT) e il tempo di protrombina (PT) negli ovini

Parole chiave
Coagulazione del sangue,
Emostasi,
Piccoli ruminanti,
Tempo di coagulazione.

Riassunto
L'emostasi è un processo fisiologico che impedisce l'eccessiva perdita di sangue. Per esaminare i sistemi di coagulazione in laboratorio si utilizzano il tempo di protrombina (PT) e il tempo di tromboplastina parziale attivata (aPTT). Quanto il sesso influenzi i valori di PT e aPTT non è noto. Il presente lavoro ha lo scopo di determinare i valori di PT e aPTT nella pecora adulta per valutare la loro relazione con il sesso dell'animale. Sono stati raccolti campioni di sangue in 40 esemplari adulti (1-6 anni) di pecore di razza mista (20 maschi e 20 femmine) tramite venipuntura giugulare, utilizzando provette contenenti 3,8% di citrato di sodio come anticoagulante. Il tempo di protrombina e aPTT sono stati determinati rilevando la formazione del coagulo. Per tutte le pecore, i valori medi di PT e aPTT sono stati rispettivamente: 7,71 ± 0,87 s e 35,7 ± 3,57 s. I valori di aPTT mostrano una differenza significativa (p = 0,0013) tra i campioni prelevati dai soggetti maschi e quelli prelevati dalle femmine, mentre la differenza nei valori di PT non è risultata significativa (p = 0,0565). Dunque, il sesso dell'animale influenza la funzione del plasma del sistema di coagulazione del sangue negli ovini. A differenza dei dati mostrati nella tabella I, in particolare, i valori dell'aPTT sono significativamente più alti nelle pecore femmine rispetto ai maschi.

Plasma blood-clotting system is a complex cascade of enzymatic reactions. Previous studies investigated the clotting profile for several species at different physiological stages (Piccione *et al.* 2008, Bazzano *et al.* 2014). Several pathological conditions, such as haemophilia A, poisoning, and infectious diseases (Neuenschwander and Pliska 1994, Van Amstel *et al.* 1994, Fernández *et al.* 1995), can influence plasma blood-clotting system in sheep. Deficiencies of clotting factors may manifest clinically as a tendency toward spontaneous bleeding, such as epitasis, melena, and haematuria (Gentry *et al.* 2008).

Clotting tests are useful for diagnosing coagulation disorders and monitoring anticoagulant therapy. Measurements of clotting parameters and platelet aggregation are performed using commercial kits within a well-defined time from blood collection (Piccione *et al.* 2010a, Piccione *et al.* 2010b). The traditional coagulation pathway is composed of the intrinsic (factors VIII, IX, XI, and XII) and extrinsic (factor VII) pathway, both of which share a common terminal pathway (thrombin and factors V and X). The main laboratory tests for clotting system are the prothrombin time (PT) and the activated partial thromboplastin time (aPTT). The PT measures the extrinsic and common terminal pathways, detecting hereditary and acquired defects in fibrinogen, thrombin, as well as factors V, VII, and X. The aPTT evaluates the intrinsic and common pathways and detects hereditary and acquired defects in prekallikrein, fibrinogen, thrombin, along to factors V, VIII, IX, X, XI, and XII (Gentry *et al.* 2008).

The present work aimed to determine the values for PT and aPTT in adult sheep and to evaluate whether these values vary with the sex of the sampled animals.

We sampled 40 adults (1-6 years old) of mixed breed sheep (20 males and 20 females). No female sheep was pregnant or showed any sign of oestrus during the study. All animals were healthy and received water and food *ad libitum*. No drugs were administered for 1 month prior to the study. Ethical procedures were based on Brazilian law[1].

Blood samples (2.5 mL) were collected via jugular venepuncture using vacuum tubes containing 3.8% sodium citrate as an anticoagulant. The citrated whole blood samples were centrifuged within 20 minutes after collection at 805 x *g* for 10 minutes at room temperature. The plasma samples were separated using plastic pipettes and transferred to plastic microtubes.

[1] Law 6638 (May 8, 1979) 'Normas para Prática Didático-Científica da Vivissecção de Animais' and 'Ethical Principles for Use of Experimental Animals' from Colégio Brasileiro de Experimentação Animal (COBEA), Brazil, which are in accordance with the 'European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes' (Strasbourg, March 18, 1986).

PT and aPTT determinations were accomplished using standard kits (Larbolab, Guarulhos, San Paulo, Brazil). For the PT assay, 100 µL citrated plasma was added to a preheated (37 °C) test tube and incubated at 37 °C for 3 minutes. Then, 200 µL preheated (37 °C) PT reagent was added, the time (in seconds) from the addition of PT reagent to the detection of clot formation was measured and defined as the PT.

For the aPTT assay, 100 µL citrated plasma and 100 µL preheated (37 °C) aPTT reagent (0.007% rabbit brain phospholipids and 0.0037% ellagic acid) were mixed in a preheated (37 °C) test tube and incubated at 37 °C for 3 minutes. Thereafter, 100 µL 0.02 M calcium chloride preheated to 37 °C was added. The time (in seconds) from the addition of calcium chloride to the detection of clot formation was measured and defined as the aPTT.

Data were compared by the unpaired Mann-Whitney test. $P < 0.05$ was considered statistically significant. Statistical analyses were performed using BioEstat 5.3. Results are presented as means with standard errors and median values.

The mean PT and aPTT for all sheep were 7.71 ± 0.14 s and 35.7 ± 0.56 s, respectively (Table I); aPTT values differ significantly ($P = 0.0013$) depending on the sex of the sampled animals, while the PT values did not ($P = 0.0565$).

Foley and colleagues report that PT and aPTT measurements in 50 healthy Samm & Border Leicester Cross ewes were 11.4-15.5 s and 19.6-40.8 s, respectively (Foley *et al.* 2014). Another study found that aPTT values in 47 German Black-Headed ewes were 33.00 ± 6.79 s (mean ± SD) (Wilhelmi *et al.* 2012). Li and colleagues also reported that the mean ± SEM of aPTT and PT in 8 healthy adult sheep (unspecified sex and breed) were 40.94 ± 3.09 s and 14.46 ± 1.52 s, respectively (Li *et al.* 2013). Thus, aPTT values herein are consistent with previous reports (Wilhelmi *et al.* 2012, Li *et al.* 2013, Foley *et al.* 2014), but PT values reported in this study are lower than those reported in the relevant literature (Li *et al.* 2013, Foley *et al.* 2014).

The evaluation of the sexual influence on coagulation in humans revealed that men presented lower prothrombin activity (Sakata *et al.* 2007) and higher concentrations of antithrombin and protein C (Dolan *et al.* 1994). In women, oral contraceptive use, pregnancy, and postpartum status increased the risk for venous thromboembolism (Chan 2010). Furthermore, men with idiopathic hypogonadotropic hypogonadism presented increased activities of factors V and X and decreased levels of antithrombin compared with healthy men (Erem *et al.* 2008). In dogs, the thrombin and factor V activities have been reported to be slightly higher in female dogs than in males (Mischke 1994).

Table I. *Values (in seconds) for prothrombin time (PT) and activated partial thromboplastin time (aPTT) in male and female sheep.*

	PT		aPTT	
	Males (n = 20)	**Females (n = 20)**	**Males (n = 20)**	**Females (n = 20)**
Mean ± SEM	8.02 ± 0.24	7.40 ± 0.11	37.7 ± 0.91	33.6 ± 0.20
Median	7.77	7.31	37.5	33.4
P[1]	0.0565		0.0013	
Both sexes	7.71 ± 0.14		35.7 ± 0.56	

SEM = Standard error of mean; [1]unpaired Mann-Whitney test.

Several hormones have been shown to influence blood coagulation. In pregnant sheep, progesterone levels had a significant interaction with PT during gestation and with aPTT on prenatal days, and estradiol-17β and cortisol were correlated with PT and aPTT values on prenatal days (El-Belely *et al.* 2000). The high serum progesterone concentrations in female sheep during the luteal phase were associated with increased fibrinogen concentration and decreased plasma antithrombin activity (Sakata *et al.* 2007). The administration of oestrogens to sheep increased the synthesis and activities of fibrinogen, thrombin, and factors VII, IX, and X (Albaker and Keeton 1982). Hyperprolactinaemia may also interfere with haemostasis. In fact, human patients with prolactinoma present increased levels of fibrinogen and tissue plasminogen activator inhibitor-1 and decreased levels of thrombin-activatable fibrinolysis inhibitor, possibly promoting a hypercoagualable state (Erem *et al.* 2010).

Based on our results, we conclude that the sex of the animal influences the function of the plasma blood-clotting system in sheep. In particular, in Table 1 the aPTT values in females were lower than in males

References

Albaker J. & Keeton K.S. 1982. Effects of oestrogen-progesterone implants on the blood coagulation system of lambs. *Am J Vet Res*, **43**, 1837-1839.

Bazzano M., Giannetto C., Fazio F., Marafioti S., Giudice E. & Piccione G. 2014. Hemostatic profile during late pregnancy and early postpartum period in mares. *Theriogenology*, **81**, 639-643.

Chan W.S. 2010. Venous thromboembolism in pregnancy. *Expert Rev Cardiovasc Ther*, **8**, 1731-1740.

Dolan G., Neal K., Cooper P., Brown P. & Preston F.E. 1994. Protein C, antithrombin III and plasminogen: effect of age, sex and blood group. *Br J Haematol*, **86**, 798-803.

El-Belely M.S., Al-Qarawi A.A. & Abdel-Rahman H.A. 2000. Interrelationship between the blood coagulation profile and plasma concentrations of progesterone, oestradiol-17β and cortisol throughout pregnancy and around parturition in sheep. *J Agr Sci*, **135**, 203-209.

Erem C., Kocak M., Hacihasanoglu A. & Yilmaz M. 2008. Blood coagulation and fibrinolysis in male patient with hypogonadotropic hypogonadism: plasma factor V and factor X activities increase in hypogonadotropic hypogonadism. *J Endocrinol Invest*, **31**, 537-541.

Erem C., Kocak M., Nuhoglu I., Yilmaz M. & Ucuncu O. 2010. Blood coagulation, fibrinolysis and lipid profile in patients with prolactinoma. *Clin Endocrinol*, **73**, 502-507.

Fernández A., Ramos J.J., Saez T., Sanz M.C. & Verde M.T. 1995. Changes in the coagulation profile of lambs intoxicated with aflatoxin in their feed. *Vet Res*, **26**, 180-184.

Foley S.R., Solano C., Simonova G., Spanevello M.M., Bird R.J., Semple J.W., Jackson D.E., Schibler A., Fraser J.F. & Fung Y.L., 2014. A comprehensive study of ovine haemostasis to assess suitability to model human coagulation. *Thromb Res*, **134**, 468-473.

Gentry P., Burgess H. & Wood D. 2008. Hemostasis. *In* Clinical biochemistry of domestic animals, 6th Ed. (J.J. Kaneko, J.W. Harvey & M.L. Bruss, eds) Academic Press, Burlington, 287-330.

Li J., Cao W., Lv X.X., Jiang L., Li Y.J., Li W.Z., Chen S.Z. & Li X.Y. 2013. Zeolite-based hemostat QuikClot releases calcium into blood and promotes blood coagulation in vitro. *Acta Pharmacol Sin*, **34**, 367-372.

Mischke R. 1994. Aktivität der gerinnungsfaktoren II, V, VII und X beim gesunden hund - abhängigkeit von alter, geschlecht und rasse. *Berl Munch Tierarztl Wochenschr*, **107**, 289-294.

Neuenschwander S. & Pliska V. 1994. Factor VIII in blood plasma of haemophilic sheep: analysis of clotting time-plasma dilution curves. *Haemostasis*, **24**, 27-35.

Piccione G., Bertolucci C., Giannetto C. & Giudice E. 2008. Clotting profiles in newborn Maltese kids during the first week of life. *J Vet Diagn Invest*, **20**, 114-118.

Piccione G., Casella S., Giannetto C. & Giudice E. 2010a. Effect of storage conditions on protrombin time, activated partial thromboplastin time and fibrinogen concentration on canine plasma samples. *J Vet Sci*, **11**, 121-124.

Piccione G., Casella S., Giannetto C., Assenza A. & Caola G. 2010b. Effect of different storage conditions on platelet aggregation in horse. *J Equine Vet Sci*, **30**, 371-375.

Sakata T., Okamoto A., Morita T., Kokubo Y., Sato K., Okayama A., Tomoike H. & Miyata T. 2007. Age- and gender-related differences of plasma prothrombin activity levels. *Thromb Haemost*, **97**,1052-1053.

Van Amstel S.R., Reyers F., Myburgh E., Pretorius G. & Sacks P. 1994. The clinical pathology of heartwater. III. Changes in blood clotting, blood calcium, blood protein, haematocrit and white-cell counts in sheep with experimentally induced heartwater. *Onderspoort J Vet Res*, **61**, 21-27.

Wilhelmi M.H., Tiede A., Teebken O.E., Bisdas T., Haverich A. & Mischke R. 2012. Ovine blood: establishment of a list of reference values relevant for blood coagulation in sheep. *ASAIO J*, **58**, 79-82.