SHORT COMMUNICATION

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Evaluation of the use of dry blood spots for progesterone determination in ewes

Avaliação do uso de manchas secas de sangue para dosagem de progesterona em ovinos

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ABSTRACT: The use of dried blood spots on filter paper has been shown to be a practical alternative in several studies with humans and animals, enabling a simple means of storing and transporting viable blood samples for various laboratory analyses. However, its applicability in the measurement of progesterone in animals is scarce. Thus, the objective of this study was to evaluate the feasibility of using dried blood spots for the measurement of progesterone in sheep. In total, 38 blood samples from 6 sheep were dripped onto filter paper, and the remainder of each sample was separated into serum. The progesterone levels in the serum samples and in the dry drops were measured by enzyme immunoassay and subsequently correlated. The levels of progesterone in the serum and dry spots showed a high correlation between the matrices (R^2 = 0.9694). In conclusion, this study demonstrated the feasibility of using samples of dried sheep blood spots for the measurement of progesterone, and the storage and transport technique can be applied in the field.

KEYWORDS: steroids; enzyme immunoassay; dried blood; sheep; filter paper.

RESUMO: O uso de manchas secas de sangue em papel filtro tem se demonstrado uma alternativa prática em vários estudos com humanos e animais, possibilitando um meio simples de armazenamento e transporte de amostras de sangue viáveis para várias análises laboratoriais. Entretanto, sua aplicabilidade na dosagem de progesterona em animais é escassa. Deste modo, o objetivo deste estudo foi avaliar a viabilidade do uso de manchas secas de sangue para a dosagem de progesterona em ovinos. Assim, 38 amostras de sangue de 6 ovelhas foram gotejadas em papel filtro e o restante de cada amostra foi separado o soro. Os níveis de progesterona nas amostras de soro e nas gotas secas foram mensurados por enzimaimunoensaio e posteriormente correlacionados. Os níveis de progesterona no soro e nas manchas secas apresentaram uma alta correlação entre as matrizes (R2=0,9694). Em conclusão, este estudo demonstrou a viabilidade do uso de amostras de manchas secas de sangue de ovino para a dosagem de progesterona, podendo a técnica de armazenamento e transporte ser aplicada a campo.

PALAVRAS-CHAVE: esteroides; enzimaimunoensaio; gota seca de sangue; ovelha; papel filtro.

INTRODUCTION

Progesterone is a reproductive steroid hormone synthesized mainly by the corpus luteum during the estrous cycle. In addition, the placenta of some animal species is also capable of producing progesterone in sufficient amounts to maintain pregnancy (SENGER, 2015). Thus, progesterone measurement is a widely used tool in the reproductive monitoring of domestic and wild animals, signaling ovarian cyclicity and the occurrence and maintenance of pregnancy (BARTLEWSKI; BABY; GIFFIN, 2011; BROWN, 2018; OLIVEIRA et al., 2018; SENGER, 2015; TSUCHIDA et al., 2022).

Blood samples and subsequent separation of serum or plasma are the best matrices for hormonal measurement; however, venipuncture can be a stressful procedure. Some alternatives, such as ear tip capillary puncture, are less invasive for use in animals, but the amount of sample obtained

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hinders the separation of serum or plasma. In addition, the difficulty in storing and transporting the samples may hinder the monitoring of animals in the field.

In humans, to minimize the volume of the collected sample and facilitate the transport of the samples, several studies have used dried blood spots on filter paper. The technique has been used to measure a variety of hormones (EDELMAN et al., 2007; SHIRTCLIFF et al., 2001; TRETZEL et al., 2014) and biological markers, such as amino acids (DIETZEN et al., 2016) and lipids (QURAISHI et al., 2006), and to perform genetic analyses (MARINO et al., 2020).

The collection of blood droplets in humans is usually performed by puncture of the capillaries of the fingertips, offering a shorter collection time, lower invasiveness, increased repeatability, the absence of postcollection processing and easier storage and transport of the samples than the traditional use of serum or blood plasma (FISHER; OBRIST; EHLERT, 2019; MCDADE, 2014).

On the other hand, despite the wide applicability of dried blood spots in humans, reports in animals are scarce. The technique has already been applied in tests for the detection of pathogens in dogs (ROSYPAL et al., 2014) and poultry sexing (SURIYAPHOL et al., 2014) and in the detection of toxic agents in dogs and birds (LEHNER et al., 2013; SHLOSBERG et al., 2011). In the area of reproduction, Sun et al. (2013) demonstrated the feasibility of using dry blood spots in the diagnosis of pregnancy in cattle when measuring pregnancy-associated plasma protein. Conversely, regarding hormonal measurement, there are reports only of the use of progesterone in pigs for the diagnosis of pregnancy (LIN et al., 1988; CHADIO et al., 2002).

Thus, considering the wide applicability of the use of dried blood spots on filter paper and the importance of hormonal monitoring for the reproductive management of domestic animals, the objective of this study was to evaluate the feasibility of using dried blood spots for progesterone measurement in sheep.

MATERIALS AND METHODS

This study was approved by the Ethics Committee on Animal Use (CEUA)/IFAM under protocol 1741.0810/2020.

A total of 38 blood samples were collected from 6 healthy and empty Santa Inês crossbred ewes aged 3 to 5 years old housed in the Sheep Sector of the Federal Institute of Education, Science and Technology of the Amazonas, East Zone campus – IFAM/CMZL, Brazil. The samples (6 to 7/animal) were collected by venipuncture of the jugular vein with intervals of 2 to 7 days between each collection to sample different phases of the estrous cycle. After blood collection, approximately 500 µl of blood was dripped onto laboratory filter paper (weight, 80 g/m^2; pore size, 14 µm) and then dried in open air (26-30°C). The remainder of each sample was transferred to a vacuum tube without anticoagulant, followed by centrifugation to obtain the serum. After drying (approximately 30 min), the filter paper was wrapped in plastic film and stored in a bag with hermetic closure. All the material (filter paper and serum) was stored at -20°C until analysis.

For the hormonal analysis, initially, the dried blood samples were standardized with the removal of six 6-mm diameter discs from the filter paper with the aid of a paper punch. The discs were placed in glass tubes containing 300 µl of deionized water, stirred, left to stand for 15 min at room temperature (21-24°C) and then centrifuged for separation of the supernatant.

The serum samples and the resuspension of dry spots were subjected to a hormonal extraction protocol with diethyl ether described by Rasmussen et al. (1996) and already used in sheep (Amaral et al., 2019). In summary, 300 µl of each sample was added to a glass tube along with 1.5 ml of diethyl ether. The material was vortexed for 5 min and centrifuged (2000 RPM, 5 min), and then the tubes were kept at -20°C for at least 2 hours. After this time, the supernatant was separated, and the extraction process was repeated with the rest of the tube material. The two supernatants from the extraction process were mixed and evaporated in a fume hood. After evaporation, 1 ml of ethanol was added, vortexed for 5 min and left to evaporate the entire content again. After this process, the material was resuspended in 300 µl of buffer solution.

Hormonal analysis was performed by enzyme immunoassay using a protocol described for several other species, including sheep (GRAHAM et al., 2001; AMARAL et al., 2019). Briefly, 96-well polystyrene microplates were labeled with an anti-progesterone antibody (CL425, Davis University - UC Davis, USA). Subsequently, the samples, calibrators and labeled hormones were added to the wells (50 µl) and incubated for two hours. After incubation, the plates were washed, the substrate solution was added (100 µl/well), and the chromogenic reaction was stopped with acid solution. The optical density of each well was measured in a microplate reader using a 450-nm filter.

The results were statistically analyzed, and the mean, standard deviation and amplitude of each group (serum and dry spot) were calculated. Then, the results were compared between the matrices (serum vs dry spot) by simple linear regression. A significance level of $P < 0.05$ was adopted.

RESULTS AND DISCUSSION

The mean progesterone levels in the serum and dry spot samples were 0.84 ± 0.87 ng/ml (0.08-3.20 ng/ml) and 0.85 ± 0.91 ng/ml (0.08-3.41 ng/ml), respectively. According to Bartlewski; Baby; Giffin (2011), the serum levels of progesterone in sheep during the estrous cycle reach maximum values close to 5 ng/ml. In turn, Amaral et al. (2019) observed serum progesterone values between 0.2 and 9.7 ng/ml throughout pregnancy in ewes. Thus, the values observed in the present study in both matrices are within those already reported for the species.

The ratio of progesterone levels in serum samples and dry spots showed a mean of 1:1.02 and a high correlation (R^2 = 0.9694; Figure 1).

Lin et al. (1988) and Chadio et al. (2002) also observed a high correlation between serum and dried blood spots levels of progesterone in pigs. Similarly, these matrices also showed a high correlation with the progesterone dosage in humans (EDELMAN et al., 2007; SHIRTCLIFF et al., 2001).

According to Gildner (2021), each drop of blood represents on average 50 µl of whole blood. However, the use of dry spot will be influenced by the absorption rate of the filter paper used, as well as by the number of discs collected from the dry blood spot (FISCHER; OBRIST; EHLERT, 2019; MCDADE, 2014). In the present study, the observed relationship between serum and dry spot close to 1 suggests that in sheep, the proposed protocol (filter paper used, number of discs collected and resuspension volume) is effective for the measurement of progesterone without the need for adaptations in the hormonal test or mathematical corrections of the values obtained, giving more practicality to the technique.

Nevertheless, some limitations of this study should be considered, such as the lack of longitudinal monitoring of a complete estrous cycle and the lack of evaluation of the impact of sample transport at different times and conditions. Thus, future studies are recommesnded to evaluate the efficiency of the laboratory technique in monitoring the entire dynamics of the estrous cycle, thus enabling the characterization of the phases of the estrous cycle through the measurement of progesterone in dry blood spots.

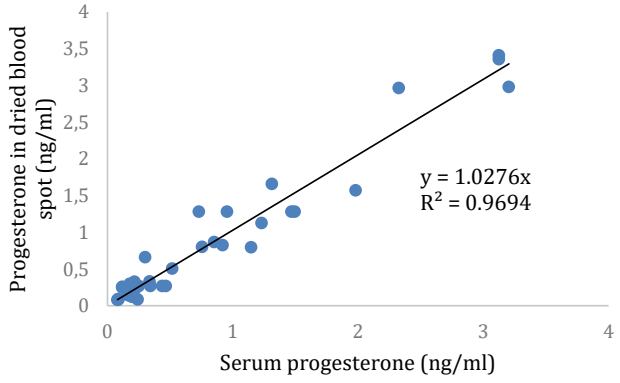

Figure 1. Linear regression of the relationship between serum progesterone levels and dry blood spots in sheep.

The effect of storage at room temperature was not evaluated in the present study because the samples of dry spots were stored at low temperatures. According to Gildner (2021), steroid hormones are usually more stable at room temperature in blood samples dried on filter paper than in serum samples, which makes it possible to transport dry samples without the need for refrigeration. On the other hand, McDade (2014) and Fischer; Obrist; Ehlert (2019) reported that more atypical conditions, such as heat and humidity, can degrade the quality of the samples, conditions that are peculiar to field conditions in warmer regions. Thus, although the literature indicates the possibility of transporting dry blood samples at room temperature, it is suggested that under field conditions, the samples should be transported in a cooled manner (in coolers), and their subsequent storage should be at low temperatures (refrigerated or frozen) to avoid the risk of degradation of biological material.

Nevertheless, the use of dry blood samples for progesterone measurement is still a useful tool, since the samples can be collected from capillaries, such as the ear tip, for example, and their storage takes up little space in the refrigerator or freezer, as well as during transport.

CONCLUSION

In conclusion, this study demonstrated, under the imposed conditions, the feasibility of using samples of dried sheep blood spots for progesterone measurement.

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