VALIDATION OF AN ENZIME LINKED IMMUNOSORBENT ASSAY FOR THE DETECTION OF PROGESTERONE IN SANTA INÊS EWES

VALIDAÇÃO DE UM ENSAIO IMUNOENZIMÁTICO PARA DETECÇÃO DE PROGESTERONA EM OVELHAS SANTA INÊS.

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The success of reproductive management in the sheep industry depends on several factors. Among them, early pregnancy diagnosis stands to improve the rates of exploration of sheep livestock, allowing the identification of fertility problems, disposal of infertile animals, food supplementation of pregnant females and maximizing production and profits. Therefore, this work aimed to develop an Enzyme Linked Immunosorbent Assay (ELISA) for detection of progesterone in peripheral blood samples from sheep of Santa Inês breed. A calibration curve was made on the test plate at which a fraction of 100 µL of diluted antigen was deposited in the wells. The antigen (P4) was diluted sample in methanol/water and incubated at 37ºC for one hour; then 200 µL were deposited from the PBS-BSA 1% and was followed by incubation overnight at 10ºC. 100 µL of anti-P4 serum produced in rabbits were added and another incubation at 37ºC for one hour was performed, followed by two washes with PBS-T-G. Then it was added 100 µL of the conjugate anti rabbit peroxidase labeled, followed by another incubation at 37ºC for one hour; two washing were done with PBS-T-G and drying the plate; revelation with TMB (Sure Blue) was performed using 100 µL of Sure Blue for 10 minutes and the reaction stopped, the plate was read at 595 nm; another revelation was done with PNPP and the plate reading was done at 450 nm. The results of the standard curve P4 are shown in Figure 1, when seen the sensitivity of the antiserum P4 was 1ng/mL for PNPP revelation, ten times more sensitive than TMB substract and standard deviation from 0 to 0.2943. Standards were performed in three replicates, to further validation test in ewe serum.
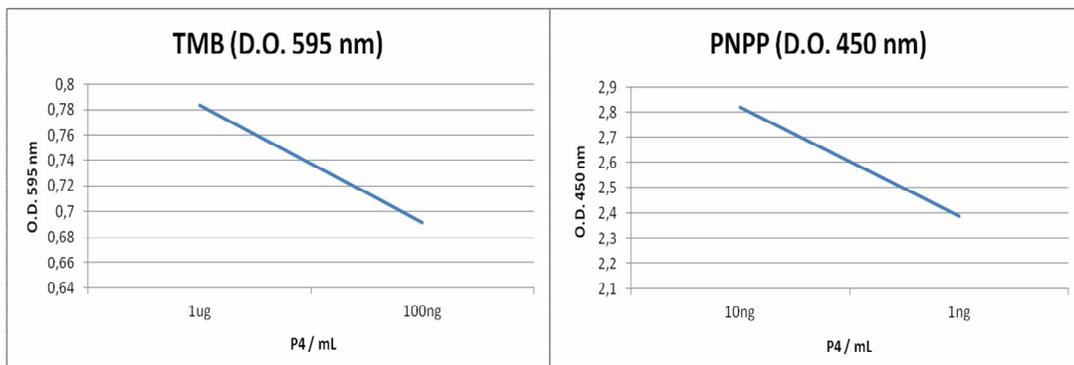

Figure 1. Titration of P4 standards, using a PTA-ELISA, with Optical Density at 595 and 450 nm.

Keywords: diagnosis of pregnancy, ELISA, ewe, progesterone.