

# Development and Validation of a Highly Sensitive Bovine Specific Enzymeimmunoassay for Determination of Plasma Kisspeptin Concentrations in Crossbred Cows

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**Abstract**—Kisspeptin (KP), a potent secretagogue of GnRH has been emerged recently as a master player in the regulation of reproduction in animals. Determination of KP in peripheral circulation is, therefore, very important for studying the control of its secretion and its role on reproduction in bovine species, the information on which is not available during any physiological state in this species, may probably be due to non-availability of simple assay procedure to measure the hormone. Therefore, the objective of this study was to develop and validate a simple and sufficiently sensitive enzyme immunoassay (EIA) for KP determination in bovine plasma using the biotin-streptavidin amplification system and second antibody coating technique. Biotin was coupled to KP and used to bridge between streptavidin-peroxidase and the immobilized KP antiserum in the competitive assay. The EIA was conducted directly in 100 $\mu$ l of unknown bovine plasma. Kisspeptin standards ranging from 0.01 to 25.6 ng/100  $\mu$ l/well were prepared in hormone-free plasma. The lowest detection limit was 0.1ng/ml plasma. Plasma volumes for the EIA, viz., 50, 100 and 200 $\mu$ l did not influence the shape of standard curve even though a drop in OD450 was seen with higher plasma volumes. Parallelism test showed good parallelism between bovine KP and KP standard curve. For the biological validation of the assay, plasma KP was measured in blood samples collected from six non-lactating cyclic cows during entire estrous cycle and from 18 pregnant cows during different stages of pregnancy. The mean plasma KP concentration during different days of the estrous cycle was different ( $P<0.001$ ). Plasma KP concentrations increased ( $P<0.001$ ) from first through last trimester of pregnancy. In conclusion, a simple, sufficiently sensitive and direct EIA procedure has been developed for the first time to determine plasma KP levels in bovine. A wide range of KP concentrations can be detected during different physiological stages in bovine using this Kisspeptin-EIA procedure.