Oxytocin release during suckling, hand-milking and machine milking managements in camels

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SUPPLEMENTARY FILE

Material and methods:

Oxytocin assay methodology :

In summary, oxytocin of the thawed serum was extracted by using a C18 liquid/solid reverse phase chromatography on single usage cartridges (Sep-Pak C18 Classic Cartridge, 360 mg Sorbent per Cartridge, 55 - 105 µm, Waters©). Eluates in methanol were vacuum dried in a speed-vac® centrifugal concentrator (Savant©), then kept at -20°C in plugged tubes to avoid oxidization until assay. Dried sample were then dissolved again in assay buffer (4 times concentration of OT) just before assay, and centrifuged again to eliminate possible precipitate of fibrin. This immunoassay is a competitive one, using a homemade tracer combining oxytocin and acetylcholine-esterase as enzyme label. We worked with a 48h pre-incubation phase between sample and antiserum and a 24h competition phase with tracer.

Standard curve was done by dilution of standard oxytocin in a pool of camel's plasma, free of oxytocin (by using charcoal fixation of small molecules) and previously extracted and reconstituted as for samples.

The Antiserum used in this assay was homemade and showed a very high selectivity with very low cross reaction to other peptides close to OT structures and other hormones susceptible to be released concomitantly and showed a specificity for the terminal tail of Oxytocin molecule recognized by receptor (Arg Vasopressin 0,3%; Lys Vasopressin 0,15%; Isotocin; 0,15%; Thr4-Gly7-Oxytocin 0,25%; Tyr-Pro-Leu-Gly-NH2 12,5%; Tocinoic Acid <7,5 10^{-5} %; Neurophysin 0,4%; ovine LH 0,15; ovine FSH 0,03%; MSH <10^{-5} %; PGF2α<10^{-5} %; ovine Prolactin 0,025 %)

Cortisol assay methodology:

For cortisol, we used the same methodology with some adjustments. Briefly, 100 µl of plasma was mixed with 1 ml of ethanol to induce protein precipitation. The supernatant was then vacuum dried in a centrifugal evaporator then reconstituted in EIA buffer (2 times

dilution). This immunoassay is a competitive one, using a homemade tracer combining cortisol and peroxidase as enzyme label. Standard curve was done by dilution of standard cortisol in a pool of camel's plasma, free of cortisol (by using charcoal fixation of small molecules) and previously subjected to alcoholic protein precipitation, supernatant drying and reconstitution as for samples.

The antiserum used in this assay was homemade and showed a very high selectivity low with low cross reaction to other close molecules or metabolites of cortisol molecule (21-deoxycortisol 4.7%, 11-deoxycortisol 4.0 %; dexamethasone 0.35%; Progesterone 0,25%; 17αHydroxy P4 0,6%; 11-deoxycorticosterone 0,43%; Testosterone 0,5%; Dehydoepiandrosterone 0,47%; Oestradiol 17β 0,08%; Cholesterol 0,5%)