應用高效液相層析法檢測牛尿中孕酮之可行性探討

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楊健仁 張維正 李善男 董嬰霎 林仁壽 吳雨新 1998。應用高效液相層析法檢測牛尿中孕酮之可行性探討。動物園學報 10:85-90。

摘要：本實驗乃嘗試利用高效液相層析法檢測牛尿中游離孕酮。其方法為以 C18 萃取管分離出尿中脂溶性物質，經 40% 甲醇預洗，消除尿中膽色素及其他可能的干擾鹽類。於 C_{18} 分離管、移動相 CH_3CN : MeOH : H_2O (1 : 1 : 1，v/v/v) 及 254 nm 紫外光波長下，在 15.4 分鐘時沖提出孕酮。本法之靈敏度達 0.25 ng/ml，而添加 5 ng/ml 及 10 ng/ml 孕酮標準品，測得之回收率分別為 70.3% 及 74.6%。

由酵素免疫分析法檢測經乙醚萃取之動情週期乳牛尿孕酮量，其濃度為 0.6 ng/ml~1.0 ng/ml，此值低於高效液相層析法所能測得之下限，故本法無法供檢測動情週期間乳牛尿孕酮之用。

關鍵字：牛尿、孕酮、高效液相層析法

前 言

應用高靈敏度放射性免疫分析法 (radioimmunoassay, RIA) 和酵素免疫分析法 (enzymeimmunoassay, EIA)，來檢測樣品中極微的孕酮含量 (~ng/ml)，已廣泛的使用在臨床及研究上，然前者有放射性的危險，後者在應用上較不普遍，兩者均需有專一性極高的抗體，且僅能估測樣品中孕酮 (progesterone) 的相對量。而利用高效液相層析法 (high performance liquid chromatography, HPLC) 則可以檢測樣品的絕對值，對反映動物生理狀況亦具參考價值 (Chang et al, 1992)。

孕酮主要由卵巢上之黃體所分泌，檢測血中之孕酮含量，可忠實地反應母畜之生殖生理狀況 (Eillicott and Dzink, 1973 ; McDonald, 1980 ; Maxson, 1987 ; Patel et al., 1995)。近十餘年來，由於野生動物保育繁殖之需，也發現糞中之孕酮與其他性類固醇含量之變化，亦能充分顯現其生殖狀態，其重要性已逐漸受到重視 (Wu et al., 1996)。然而尿中之孕酮含量如何？除少數幾篇有關人類之研究外 (Chattoraj et al., 1996 ; Stanczyk et al., 1997)，動物方面未見報告。

本研究之目的，在探討以 HPLC 檢測乳牛尿中游離態孕酮之可行性，期能進一步對於動物園動物提供另一種非侵入性的生理檢測方法。

材料與方法

一、材料

(一) C_{18} 萃取管 (Waters, part #51910)
(二) 甲醇 (皓峰)
(三) 抽氣萃取裝置 (Supleco 廠牌，日製)
(四) HPLC 設備
本研究使用之設備為日立 Model L-4000

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Quantitation of bovine urinary progesterone by using high performance liquid chromatography.

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Abstract: The progesterone content in tissue fluids and excreta can be a reliable marker for monitoring ovulation and corpus luteal function in animals. The immunoassay, such as radio immunoassay (RIA) and enzyme immunoassay (EIA) with the high sensitivity is commonly used for detecting progesterone levels. However, the radioactive hazard caused by the former restricts its usage, and the latter is limited to a few laboratories. High performance liquid chromatograph (HPLC) method measured the absolute value of steroids may provide an alternative choice.

A rapid, sensitive method of HPLC to measured free progesterone in bovine urine without complex preparing procedure was developed. One ml of urine sample was extracted by C18 cartridge, then 40% methanol was added to expel the interfered substances from urine. Progesterone in the eluent was determined by reverse phase HPLC. The results revealed that : retention time, 15.4 min; the sensitivity of urinary sample was 0.25 ng / 20 ul with the condition of 254 nm uv wavelength, a mobile phase of acetonitrile / methanol / water (1:1:1,v / v / v) and a flow rate of 0.8 ml / min. and the recovery rates were 70.3%, 74.6% for 5 ng / ml P_4 and 10 ng / ml P_4 respectively.

This HPLC system for urinary progesterone determination was unable to detect the urinary free progesterone of estrous dairy cow, due to the low concentrations. After ether extract urinary sample, the free progesterone were between 0.6 ng / ml~1.0 ng / ml, lower than the sensitivity of the HPLC system.

Key Word: Cow urine, progesterone, HPLC

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