

**Abstract:**

A simple and rapid (less than 2 h) immunoassay method has been developed based upon a novel separation technique called LIDIA (Ligand Differentiation Immunoassay), enabling direct estimation of the concentration of oestrone sulphate in ethanolic extracts of blood plasma. An antiserum raised against oestrone-3-glucuronyl-BSA was used which showed a higher cross-reaction with the sulphate than the glucuronide metabolite. The assay had a sensitivity of 5.2 pg/tube and acceptable inter-(less than 18%) and intra-(less than 8.5%) assay precision. Analysis of samples of peripheral venous plasma obtained daily from Pony mares showed that the mean concentration of oestrone sulphate started to rise from a baseline value (less than 300 pg/ml) at 6 days and reached a peak (greater than 850 pg/ml) at 2 days before follicular rupture as determined by rectal palpation. Progesterone concentrations only started to rise above baseline (less than 0.5 ng/ml) on the day of ovulation and reached a peak 8 days later. Analysis of samples obtained during the first 30 days of pregnancy showed that there was no increase in oestrone sulphate at the time oestrus would have been expected had the mares not conceived.