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This Week's Citation Classic

Neill J D, Johansson E D B, Datta J K & Knobil E. Relationship between the plasma levels of luteinizing hormone and progesterone during the normal menstrual cycle. *J Clin. Endocrinol. Metab.* **27:**1167-73, 1967.
[Dept. Physiol., Univ. Pittsburgh Sch. Med., Pittsburgh, PA]

A rapid and relatively simple competitive protein binding assay for the steroid hormone, progesterone, was developed and applied to the measurement of plasma progesterone levels daily throughout the human menstrual cycle. [The *SCI*® indicates that this paper has been cited over 365 times since 1967.]

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"I joined the laboratory of Ernst Knobil, department of physiology, University of Pittsburgh, as a postdoctoral fellow in the Fall of 1965 to study the hormonal control of the menstrual cycle in the rhesus monkey. These studies required measurement of circulating hormone levels so I visited A. Rees Midgley, department of pathology, University of Michigan, to learn the radioimmunoassay for human luteinizing hormone (LH) that he had recently developed[1] (in fact, when I visited, his method had been published only in abstract form). Next we turned to the measurement of progesterone. All of the available methods were extremely laborious, lacked sensitivity, and required the expertise of an organic chemist. Fortunately for us, Beverly Murphy published an abstract in the Spring of 1966 describing a simple and highly sensitive assay for a related steroid hormone, cortisol, based on the 'competition' between radioactive and non-radioactive cortisol for binding to a plasma protein, corticosteroid binding globulin (CBG or transcortin).[2] Our initial plan was to enzymatically convert progesterone, isolated from plasma, to cortisol and then use her method for quantification. However,when we phoned her to get additional details about the method, she told us that progesterone itself also bound to CBG and could be measured directly without the conversion step. I added a thin-layer chromatographic step to her method so that it would be specific for progesterone, and on July 4, 1966, obtained the first convincing evidence that the method could be applied to measurement *of* progesterone in plasma.

"Because the human LH radioimmunoassay turned out to be unsuitable for measuring monkey LH, we studied the human menstrual cycle by collecting daily blood samples from four women for simultaneous measurements of progesterone and LH (of the group of co-authors, only Johansson was a physician). Ours and Murphy's manuscripts were submitted simultaneously for publication with instructions to the editor that our paper should appear one month after Murphy's, and it did.

"This paper was cited frequently for several reasons. First, it ushered-in an era of relatively rapid and simple measurements of plasma steroid hormone levels; indeed, many people visited our laboratory to learn the method long before it was published. Second, and of more lasting significance, it established unequivocally and for the first time the relationship between the secretion of progesterone and LH. At that time, progesterone was believed to be the ovarian stimulus for the increased LH secretion that results in ovulation. Our paper showed that progesterone secretion increased only after the increase in plasma LH and hence was not the ovarian signal. It also established that the regression of the corpus luteum —as signified by a decrease in plasma progesterone levels —was not associated with changes in the secretion of its luteotrophic hormone, LH."

1. **Midgley A R, Jr.** Radioimmunoassay: a method for human chorionic gonadotropin and human luteinizing hormone. *Endocrinology* **79:**10-18, 1966.
2. **Murphy B E P.** Some studies of the protein-binding of steroids and their application to the routine micro and ultramicro measurements of various steroids in body fluids by competitive protein binding radioassay. *J Clin. Endocrinol Metab.* **27:**973-90, 1967.