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This Week's Citation Classic

Johansson E D B. Progesterone levels in peripheral plasma during the luteal phase of the normal human menstrual cycle measured by a rapid competitive protein binding technique. *Acta Endocrinol.* **61**:592-606, 1969.
[Department of Obstetrics & Gynecology, University of Uppsala, Uppsala. Sweden]

A rapid method for the estimation of progesterone in plasma is described. After a simple extraction with petroleum ether, progesterone is quantified by competitive protein binding. The catch was that the batch of petroleum ether had to have certain properties to avoid crossreacting steroids. [The *SCI*® indicates that this paper has been cited over 265 times since 1961.]

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"In the summer of 1967 Jimmy Neill, Ernst Knobil, J.K. Datta and myself had published a method for progesterone based on the pioneer work on competitive protein binding by Beverly Murphy.[1,2] I had the privilege to come into this work as a postdoctoral fellow at the department of physiology in Pittsburgh (Ernst Knobil).

"When I got back to the department of obstetrics and gynecology at the University of Uppsala, Sweden, I immediately started to assemble equipment and to train personnel to get the method started. I had lots of projects, and my chief, Carl Gemzell, gave me constant encouragement.

"The method contained a tedious thin layer chromatography step. This gave me the biggest problem and I often had to do the chromatography myself. On the morning of February 2nd I was very tired and very happy. My daughter had been born at 4 o'clock in the morning. I went down to the lab and started to work with the assay. I had always tried to get rid of the thin layer step. I had tried it in Pittsburgh with rhesus monkey plasma but it did not work. This morning I decided on another try to omit the thin layer step. I was simply too tired to do it. When the assay was done I regretted it. What a waste of time, I thought. The next morning when I calculated the results my heartbeat accelerated. The pool samples were almost identical to the results from the thin layer assays. Why? It turned out that the lot of petroleum ether that I used extracted progesterone well, but only minute amounts of cortisol and 17α-hydroxy-progesterone, the main crossreacting steroids in the quantification step.

"I spent the following months trying to prove that progesterone in women could be measured with this rapid technique. The method worked very well in our hands. With the same manpower as before we could describe the pattern of progesterone during the menstrual cycle and normal pregnancy and start mode of action studies on hormonal contraceptives. Family planning was and is my main interest. That was why I started to do research.

"Now a days the assay methods using competitive protein binding have been replaced by radioimmunoassays, which are even more rapid, sensitive, and specific.

"There are several reasons for the many citations of this paper. First of all it describes a new method. The method was so simple that it attracted the interest of people who usually at that time would not think of steroid measurements. The paper also presented daily plasma levels of progesterone from 20 ovulatory menstrual cycles. This was a quantity of steroid measurements never seen before. It attracted gynecologists, veterinarians, and physiologists who saw new possibilities for studies in their own field. The third explanation for its many citations is my own frequent citation of the paper."

1. **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.
2. **Murphy B E P.** Some studies of the protein-binding of steroids and their application to the routine micro and ultramicro measurement of various steroids in body fluids by competitive protein-binding radioassay. *J. Clin. Endocrinol. Metab.* **27**:973-90, 1967.