

# "Biochemical Studies on the Local Production of Radioimmunoassay System Components for Serum Progesterone"

Thesis

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## **Abstract**

Measurement of P<sub>4</sub> in serum is an important tool for the diagnosis of ovarian dysfunction and precise timing of ovulation in women and detection of normal luteal function which may combine with infertility and early miscarriage.

The preparation of high technology RIA reagents with low cost is considered to be one of the important goals of the present study. The development of liquid phase radioimmunoassay for the measurement of P<sub>4</sub> in human serum is described.

### **The present work achieved:**

- The preparation of (P<sub>4</sub>: BSA) immunogen.
- The production of P<sub>4</sub> polyclonal antibodies, P<sub>4</sub> standards and radiolabeled P<sub>4</sub> tracer.
- Establishment of local radioimmunoassay method (liquid phase RIA technique).
- Optimization and validation studies of the local assay were carried out using the performance characteristics of the succeeded immunoassays.

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## Abbreviations

P <sub>4</sub>	Progesterone
P <sub>4</sub> -HS	Progesterone hemisuccinate
LH	Lutenizing hormone
hCG	human Chronic Gonadotropins
CBG	Corticosteroid binding globulin
RIA	Radioimmunoassay
IRMA	Immunoradiometric assay
FSH	Follicular stimulating hormone
LRF	Lutenizing realizing factor
E <sub>2</sub>	Estradiol
GnRH	Gonadotropin releasing hormone
ANS	8- anilino-1- naphthalene sulfonic acid
CEA	Carcinoembryonic antigen
DHEA-S	Dehydroepiandrosterone
T <sub>3</sub>	Triiodothyronine
T <sub>4</sub>	Thyroxine
Ab	Antibody
Ag	Antigen
B	Bound
B <sub>0</sub>	Maximum binding
MAbs	Monoclonal Antibodies
BSA	Bovine serum albumin
sIgM	Immunoglobulin M
C.V	Coefficient of variation
Ch.T	Chloramine- T
Ci	Curie
Cpm	Count per minute
TA	Total activity
NSB	Non specific binding
LPS	Lipopolysaccharide
FAC	Freund's Adjuvant Complete.

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FCI	Freund's Adjuvant Incomplete.
HAT	Hypoxanthine aminopterin and thymidine
PEG	Polyethylene glycol
TSH	Thyroid stimulating hormone
Ka	Affinity constant
HFS	Hormone free serum
ACTH	Adrenocorticotrophic hormone
S.D.	Standard deviation
MDC	Minimal detectable concentration
HPLC	High performance liquid chromatography
DPC	Diagnostic Products Corporation
DDW	Double distilled water
THF	Tetrahydrofurane
Ka	The association rate constant of affinity
r	Correlation coefficient

## **Introduction**

Progesterone (4-pregnene-3,20- dione) is a steroid hormone plays an important role in the preparation for and maintenance of pregnancy. As with other steroids, progesterone is synthesized by the adrenal cortex in both men and women (*Carl and Edward, 1994*).

Progesterone is secreted in very small amounts by the ovaries during the follicular phase of the menstrual cycle. The rate of secretion increases sharply during the luteal phase of menstrual cycle, reaching its maximum 5-10 days after the midcycle LH surge. Unless pregnancy occurs, a steep decline to follicular levels sets in about 4 days before the next menstrual period. This pattern constitutes the rationale behind the well- established use of serum progesterone measurements as a simple and reliable method for ovulation detection. If fertilization has occurred, the corpus luteum continues to secrete massive amounts of progesterone until the 12<sup>th</sup> week of pregnancy. The placenta then takes over and becomes the principle site of production of the hormone (*Nulsen and Peluso, 1992*).

The combined effect of progesterone and estradiol is to cause the endometrium to change from the proliferative to the secretory phase which favors the implantation of the fertilized ovum. Progesterone has multiple other actions in female reproductive physiology, including participation in feedback regulation of pituitary gonadotropins and ovarian estrogens, stimulation of mammary gland maturation during lactation, maintenance of the vaginal epithelium, uterine smooth muscle relaxation during embryogenesis and stimulation of basal body temperature (*Melzger, 1992*).

Serum levels of progesterone are relatively high at birth due to placental production, fall rapidly during the postnatal week and rise during puberty. Serum progesterone levels in adult men and postmenopausal women are similar to levels in women during the follicular phase of the menstrual cycle and reflect adrenal progesterone production (*Wood et al., 1985*).