Chapter 5

## CONCLUSION

A specific radioimmunoassay method has been standardized for assaying the serum progesterone levels. The specific antibody was kindly supplied from Dr G. Mikhail, which was prepared in ewes using a 11$\alpha$-hydroxyprogesterone hemisuccinate derivative conjugated to crystalline bovine serum albumin. The specificity studies of the antiserum indicated that the antiserum was specific for progesterone since no cross-reaction occurred with the major known steroids such as 17-hydroxyprogesterone and 20-dihydroprogesterone which have been shown to be a strong binder for the cortisol binding globulin. The precision of the assay was satisfactory since both the within and between-assay coefficient of variation was less than 10%. The accuracy of the method showed that for any samples containing more than 300 pg/ml of progesterone up to 5,000 pg/ml, the method was reliable with the recovery of more than 80%. The sensitivity of the method was in the range of 10-25 pg/tube which was satisfactory for the present study.

The effect of time and temperature of incubation on the assay were studied. It was found that when the antibody was used at a final dilution of 1:28,000, the procedure could be carried out at a temperature from 4°C up to 37°C and a minimum effective incubation time was 4 hours. However, for convenient sake, 4°C overnight incubation was used. The 0.6% charcoal was found to be the minimum and suitable concentration of charcoal for separating the free from bound steroids.

Determination of serum progesterone in four Thai normally menstruating subjects showed that the levels of progesterone were similar to those observed in Caucasian women, ranging from 0.48 to 0.60 ng/ml during the follicular phase and 2.04 to 13.50 ng/ml during the luteal phase of the menstrual cycle.

In another group of subjects who were using Norgestrel - the continuous low dosage progestational drug - as a contraceptive pill. All of these subjects, except one, had low progesterone levels in the follicular phase during Norgestrel treatment. Only one subject had progesterone levels suggesting ovulation and functioning corpus luteum. This data was different from similar studies in Caucasian women where there was a rise in progesterone level in at least 40-50% of treated cycles indicating ovulation and corpus luteum function.

Twenty-nine pregnant subjects at various stages of gestation showed a gradual and progressive rise of serum progesterone with a plateau around the end of the gestation period (levels being 17.20±6.24 ng/ml at 4-8 weeks of gestation, 62.00±36.10 ng/ml at 16-28 weeks, 169.48±52.71 ng/ml at 32-38 weeks and reaching 177.76±4.36 ng/ml at 39-40 weeks of gestation).

The study of nine subjects during the postpartum period showed that the levels of serum progesterone decreased rapidly from 19.19 ng/ml at 17 hours to 1.65 ng/ml at 116 hours after the delivery.