大韓不妊學會雜誌 : 第3卷 第2號 1976
Korean J. Fertil. Steril., Vol. 3, No. 2, Dec.

Plasma Progestin Concentration In Artificially Maintained Pregnancy

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=국문초록=

인공적으로 임신을 유지시킨 동물에 있어서 Plasma Progestin의 농도

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동물에서 정상적인 임신유지를 함에 필요한 홀몬의 양을 측정하기 위하여 토끼에서 인공수정을 실시한 다음날 양쪽 ovary를 제거하고, 홀몬대치요법(replace therapy)에 의해서 인공적으로 임신상태를 분만시까지 유지시키면서 competitive protein-binding assay method을 이용하여 혈중 progestin의 농도를 측정하여 정상 임신토끼와 비교하였던 바 다음과 같은 결과를 얻었다.

1) 정상임신토끼에 있어서 혈중 progestin의 평균 농도 변화는 제1일에 1.7~2.7 ng/ml으로 시작해서 차차 증가되어 제13~15일까지는 19.8~25.3 ng/ml까지 상승하였다가 그후 분만시까지 서서히 감소되었다.

2) 홀몬 대치요법에 의해서 인공적으로 임신을 유지시킨 토끼의 혈중 progestin농도는 정상토끼의 progestin 농도의 변화와 매우 비슷한 변화곡선을 나타내었다.

Introduction

No systmeatic study has, as yet, been reported on the changes in the concentration of progestin in the plasma of the artificially maintained pregnancy of the rabbits. While the present experiments were being conducted, experiments on the concentration of progesterone and oestrogens in the peripheral plasma of rabbits during the course of pregnancy were reported using radiommunoassay (Challis, Davies and Ryan, 1973) or competitive protein binding techniques (Baldwin and Stabenfeldt, 1974). The progesterone profiles in these two papers showed a slow increase after mating, reached peaks around midpregnancy and decreased thereafter until Day 32. These findings agreed with some of the earlier ones (Mikhail, Noall and Allen, 1961; Okano, Matsumoto, Kotoh, Endo and Seki, 1966; Hilliard, Spies and Sawyer, 1968).

In the previous work (Kwun and Emmens, 1975), systematic dose levels of progesterone and oestradiol which, on their own, could support normal implantation and pregnancy to term were established by replacement therapy in rabbits ovariectomized the day after mating.

In the present experiments, blood progesterone levels were checked by direct measurement, from the time of mating through to parturition in normal pregnancy, and in artificially maintained pregnancy in ovariectomized rabbits. This was carried out in order to estimate the actual endocrine requirements for implantation and maintenance of pregnancy to term, and to see how closely the empirically determind replacement therapy copied the results from natural blood studies.

Materials and Methods

Sexually matured virgin albino rabbits (mean body weight±S.D. : 2.83±0.40 kg), aged 6 to 7 months, were kept in individual wire cages. Animals were isolated for at least 2 weeks before the start of an experiment. They were injected with H.C.G. and inseminated on Day 1, and followed by ovariectomy on Day 2 as by Kwun and Emmens (1975). The injection schedule of progesterone and oestradiol was same as the replacement theraphy of Kwun and Emmens (1975).

The blood samples obtained by cardiac puncture were immediately cooled to 5°C and the plasma separated by centrifugation at 1500 g at 4°C for 15 minutes within 2 hours after collection. The plasma was stored at −20°C until an analysis.

All new glassware was soaked in R.B.S. detergent solution (Borghgraef, Belgium) overnight, washed with warm tap water and soaked again in concentrated H_2SO_4 overnight. The acid washed away throughly with tap water and double distilled water and the glassware then dried. Finally, the glassware was rinsed with ethanol (A.R.) and dried again. The used glassware was rinsed with acetone and then soaked in the R.B.S. solution and

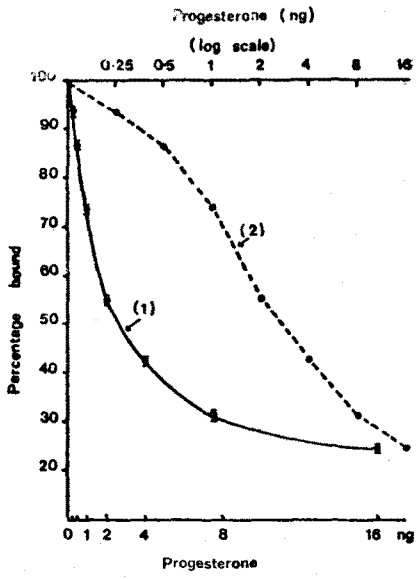

Fig. 1. Standard curve for progestin measurements from 12 routine standard curves for assay method B

Each point indicates mean±S.E. (n=12)
(1): Progesterone standard curve
(2): Progesterone standard curve on a long scale

subjected to the same process of washing as above. No plastic materials were used in the assay system.

The concentration of progestin in the plasma was determined by the competitive protein-binding method described previously by Johanson (1969), with some modifications.

The standard courves obtained using progesterone binding globulin (PBG) from pregnant guinea pig (52 days gestation) (Tan and murphy, 1974) and labelled progesterone as a competing substance were found to be sensitive and highly reproducible (Fig. 1). The recovery rate of progesterone by hexane extraction was 93.5% (n=5). A quantity of 0.1 ml of dichloromethane was added in the process of extraction of the plasma samples to remove any steroid which had been absorbed to the inside of the extracting tube wall.

In fourteen consecutive standard curves over

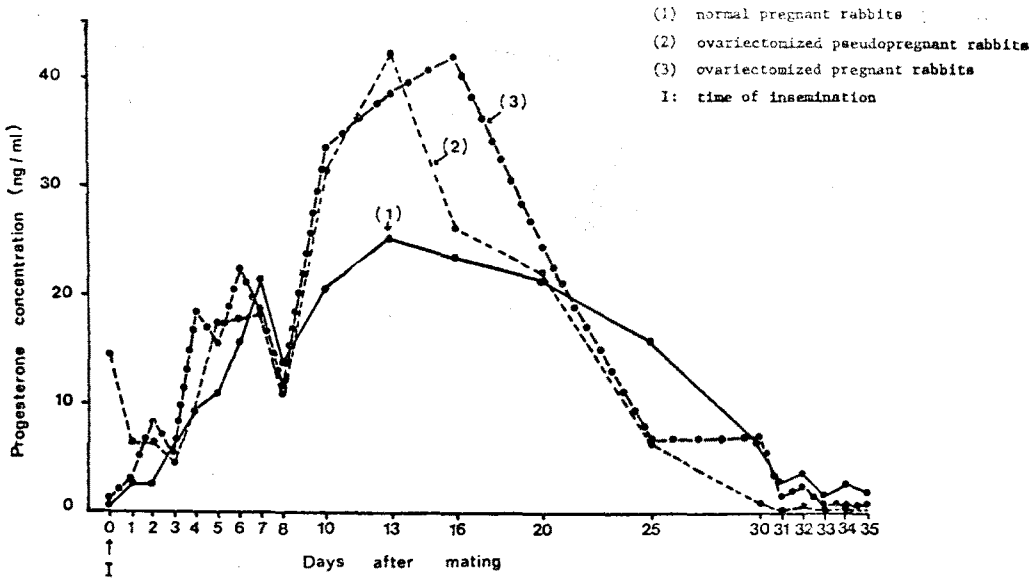

Fig. 2. Comparison of plasma progestin levels between normal pregnant rabbits and ovariectomized Pseudopregnant and pregnant rabbits injected with exogenous steroids (Experiment 5. 5)

the range 0. 25 to 16. 0 ng of progesterone the coefficient of variation (CV) of duplicates was 6. 2%, and the coefficient of variation within assays for progesterone in 0. 5 ml of rabbit plasma samples in 20 duplicates was 8. 3%. The data presented in this experiment were not corrected for extraction losses of 6. 5%. The assay data were analyzed by the computer program of Rodbard and Lewalt (1970).

Results

The levels of progestin found in normal pregnant rabbits and in rabbits under artificially maintained pregnancy by replacement therapy are given in Figure 2. The progestin leveals prior to insemination averaged 1. 3 ng/ml of plasma. The mean concentration of progestin rose from 2. 72 ng/ml on Day 1 to 25. 28 ng/ml on Day 13. Thereafter the mean concentration decreased gradually to 2. 96 ng/ml on Day 31 and to 1. 4 to 2. 45 ng/ml postpartum. There was a peak around the time of implantation, on Day 7. The progestin concentration of 21. 36 ng/ml on Day 7 was significantly higher than that of 13. 96ng/ml on Day 8 ($t=2.41$, $p<0.05$). The actuaul concentrations of plasma progestin agree well with those reported recently by Challis et al. (1973) and by Balawin and Stabenfeldt (1974).

Progesterone levels in the artifically maintained pregnant rabbits followed quite well the normal levels until Day 8 but they increased abruptly to 34. 0 ng/ml on Day 16. Following this peak, levels decreased rather quickly to 6. 9ng/ml on Day 25 and to near blank values on Day 35. In these animals, treated with 6 mg of progesterone plus 0. 2 ug oestradiol from Day 9 to Day 19, the mean progesterone level on Day 13 was significantly higher than that of normally pregnant rabbits ($t=2.59$, $p<0.05$). When treatment was dropped to 3 mg and down to 1 mg of progesterone plus 0. 2 ug oestradiol from Day 20 to Day 29, the mean progesterone level on Day 25 was significantly lower than that of normal pregnant animals

($t=4.28$, $p<0.01$).

In the pseudopregnant rabbits ovariectomized the day after mating, which were given replacement therapy as for the pregnant ovariectomized rabbits, the concentration of plasma progestin prior to insemination was 1.4 ng/ml. It declined to 6.3 ng/ml on Day 1. Thereafter, the progesterone profile was similar to that in the artificially maintained pregnant rabbits. However, the peak levels were reached 3 days earlier and they declined earlier than those of the artificially maintained pregnant rabbits.

Discussion

Progesterone levels in Fig. 2 remained low on Days 1 and 2 and rose from Day 3, reaching a peak level on Day 13 after mating, followed by a gradual decline between mid-pregnancy and term. During the period of tubal transport, steroid output remained very low and it might seem likely that itis not very important. Yet without administration of progesterone on Days 2 to 4 no implantation occurred (Kwun and Emmens, 1974).

The progestin profile in peripheral plasma during pregnancy in Fig. 2 is similar to those reported by other workers (Challis et al., 1973; Baldwin and Stabenfeldt, 1974), and only slight differences exist in the absolute quantities of progestin reported to be present in plasma. Challis et al. (1973), using radioimmunoassay, found progesterone concentration of 17 to 19 ng/ml on Days 12 to 15. Using the competitive protein-binding assay, Baldwin and Stabenfeldt (1974) reported peak progesterone levels of 13.3 ng/ml on day 18, and Kendall and Liggins (1972) have reported a progesterone concentration of 25 ng/ml on Day 20 of pregnancy. These are all in good agreement with the present experimental results. The progestin declined prior to parturition in the rabbit is similar in most species in which pargnancy maintenance depends only upon the corpora lutea (Davies and Ryan, 1972).

When pseudopregnancy was induced by injection of HCG, the initial peak level of preovulatory progestin was significantly lower than in normal pregnant animals (Fig. 2). The level was low at the onset of pseudopregnancy, rose to a peak on Day 10 and gradually declined towards the end of the period. In pregnant rabbits, the rise in plasma progestin occurred at an earlier stage than in pseudopregnant rabbits. On Day 6, plasma progestin levels were significantly higher in the pregnant animals (11.8 ng/ml) than in the pseudopregnant ones (4.0 ng/ml) than in the pseudopregnant ones (4.0 ng/ml). This is in good agreement with the results of Beling, Fuchs, Hour, Park and Sexena (1974), who suggested that production of substances by the foetus may exert a maternal luteotrophic influence before implantation takes place on Day 7. In the pseudopregnant rabbit, it was reported that progesterone secretion by the ovary rose to a peak at Days 7~9 then gradually declined, but the level of 20α-hydroxyprogesterone increased towards the of pseudopregnancy (Hilliard et al., 1968; Horrell, Major, Kilpatriok and Smith 1972).

The progesterone profile of peripheral plasma in artificially maintained pregnancy showed quite a similar pattern to that in normally pregnant rabbits. But it showed levels of progestin during mid-pregnancy higher than, and after Days 20 to 29 of gestation levels lower than, those in normalls.

In such comparison of steroid levels between normally and artificially, it must be taken into consideration that the time of steroid injection and the timing of bleeding after the injection

may influence results. In addition, some possible additive effects or accumulation of the steroids injected twice daily have to be considered in such experiments.

The result of this experiment seems to indicate that the administration of 6 mg/day of progesterone during Days 9～19 was some what too high, and the dosage of 3mg/day of progesterone during Days 20～25 was too low compared with the normal progestin secretion rate in pregnancy. Therefore, even during mid-pregnancy no more than perhapes 4 or 5 mg of progesterone per day is needed to maintain pregnancy.

The values obtained in these experiments for peripheral plasma progestin in normally pregnant rabbits included both progesterone and 20α-hydroxyprogesterone. Moreover, 20α-hydroxyprogesterone appears to have a special functional significance in the rabbit. Even in the absence of progesterone, 20α-hydroxyprogesterone output is rapidly accelerated following coitus, and during the preovulatory period several milligram are released into the circulation (Hilliard, Hayward and Sawyer, 1964). Therefore, for a better understanding of the function of ovarian steroids in the rabbit, especially the preovulatory steroid secretion by the ovary, a separate measurement of progesterone and 20α-hydroxyprogesterone is really needed.

Summary

The changes of the plasma progestin concentations in normal pregnancy and artificially maintained pregnancy of the rabbits by hormonal replace therapy after induced ovulation and insemination on Day 1, followed by ovariectomy on Day 2, were checked by direct measurement, competitive protein-binding assay, from the time of mating through to parturition. The results obstained in this experiment can be summarized as follows:

(i) The mean concentration of progestin in normally pregnant rabbits increased from 1.7～2.7 ng/ml on Day 1 to 19.8～25.3 ng/ml on Days 13～15 and declined slowly thereafter until term.

(ii) The progesterone levels in artificially maintained pregnancy by hormonal replacement therapy showed quite a similar pattern to that in normally pregnant rabbits.

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