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Studies on the improvement of reproductive efficiency in Korean native cows
—Development of radioimmunoassay for progesterone—

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韓牛의 繁殖效率 增進에 관한 硏究
—Progesterone測定을 위한 放射線免疫分析法의 開發—

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초록 : 항체로서 4-pregnen-11α-ol-3,20-dione-hemisuccinate bovine serum albumin을 사용하여 progesterone 측정을 위한 방사선 면역분석법을 개발하였다. 항체의 최종 희석 배율은 1 : 5,000이었다. Label progesterone의 회수율은 84%였으며 최저 측정치는 8pg/ml이었다. Intra- and interassay coefficients of variation은 각각 6.2, 10.4%이었다. 방사선 면역분석법은 가축에서 性成熟, 發情確認, 妊娠診斷, 分娩후 卵巢機能回復 및 繁殖障碍의 診斷등에 적용될 수 있을 것으로 사료된다.

Key words: progesterone, cross-reaction, antibody, Korean native cow.

Introduction

Korean native cattle (*Bos taurus coreanae*) has been an important livestock in Korea not only for meat production but also for farming. Low production efficiency in Korean native cattle means less calves per year per cow and number of cows to be hold a farm is only one or two cows by cultural tradition. The Korean native cattle is to reach puberty near 24 months of age.[1,2] But the reports[3,4,5,6] have wide variations, so this needs to be objectively determined. Estrus detection is a serious problem for breeding programs.

The accurate detection of estrus is paramount in ensuring optimum fertility. Poor detection is probably the most important reason affecting delayed breeding. Almost 30% of the Korean native cows have some problems in estrus detection. With poor estrus detection in Korean native cows, postpartum period lasts 5 to 6 months which results in an extended calving interval and delayed breeding.[1,6,7] There are great variations among individual cow in the intensity of heat signs depending on the level of nutritional intake. Chung et al[8] reported that the first estrus in the heifer fed a nutrious diet appeared at 14.6 months when body weight was 265kg. Age at first calving was an average 28.9 months with a body weight of 436kg and the first estrus was observed at 37.8 days postpartum in the group, whereas there was delay in first ovulation and estrus in the restricted feeding heifers (73.4 days and 111.8 days).[9]

The purpose of the present study is to develop a

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sensitive and specific radioimmunoassay for progesterone and to measure plasma concentrations of progesterone during the estrus cycle in Korean native cow.

Materials and Methods

Heparinized blood samples taken by jugular venipuncture were centrifuged immediately(15min., 600g) and the plasma stored at −20°C until used for the determination of progesterone.

The RIA for progesterone was carried out as follows. The steroid was extracted from the sample (0.2ml) with 5ml of petroleum ether(40∼60°C bp) after addition of 100μl [^{3}H] progesterone(2,000 disintegrations/min; NET-381, New England Nuclear, Dreieich, West Germany) in 0.05ml of a solution of 0.1M-phosphate buffer, pH 7.0, containing 9.0g of sodium chloride, 1.0g of sodium azide and 1.0g of gelatine per litre of solution(G-PBS). After extraction for 20 minutes on a mechanical shaker the aqueous phase was frozen during centrifugation at −20°C, after which the organic phase was transferred to another tube and dried down under a stream of nitrogen. The residue was then redissolved in 2ml G-PBS.

For the RIA for progesterone 0.1ml of the sample and steroid standards were incubated overnight at 4°C together with 0.1ml of antiserum(final dilution 1 : 5,000, v/v), which were developed in goats using progesterone conjugated with BSA, and 0.1ml of [^{3}H] progesterone(20,000 disintegrations/min).

Free and bound steroids were separated by dextran-coated charcoal(0.5ml of a suspension of 0.6% charcoal and 0.06% dextran T-70 in G-PBS). The radioactivity of the antibody-bound steroid was measured in a liquid scintillation counter and the amount of hormone in the sample estimated from a standard curve. After correcting for recoveries the results have been expressed as ng per ml of sample. All samples were assayed in duplicate.

To investigate the progesterone concentrations in 10 Korean native cows with a normal estrous cycle, blood samples were taken on alternate days for 2 estrous cycles. Plasma progesterone concentrations were measured by a radioimmunoassay as descrived above.

Results and Discussion

The final dilution of antibody was 1 : 5,000 for progesterone. The cross-reactions as calculated at 50% inhibition of binding were tested with the following steroids: 5-pregnen-3β-ol-20-one, 4-pregnen-3,20-dione, 4-pregnen-17α-ol-3,20-dione, 4-androsten-3,17-dione, 4-androsten-17β-ol-3-one, 4-androsten-17α-ol-3-one, 5-androsten-3β-ol-17-one, 5-androsten-3α,17β-diol, 5-androsten-3β,17β-diol, estrone, estradiol-17α, estradiol-17β and estriol.

The antiserum for progesterone showed cross-reactions with 4-pregnen-3,20-dione (100.0%), 4-pregnen-17α-ol-3,20-dione (0.1%), 4-androsten-3,17-dione (0.1%), 4-androsten-17β-ol-3-one (0.1%), 4-androsten-17α-ol-3-one (0.1%), 5-androsten-3β-ol-17-one (0.1%), 5-androsten-3α,17β-diol (0.1%), 5-androsten-3β,17β-diol (0.1%), estrone (0.1%), estradiol-17β (1.1%) and estriol (0.1%).

The graph shown in Fig 1 is a standard curve where the percentage bound tracer against the concentrations of the steroid standard: 8, 16, 31, 62, 125, 250 and 500 pg per ml is on a linear scale. The

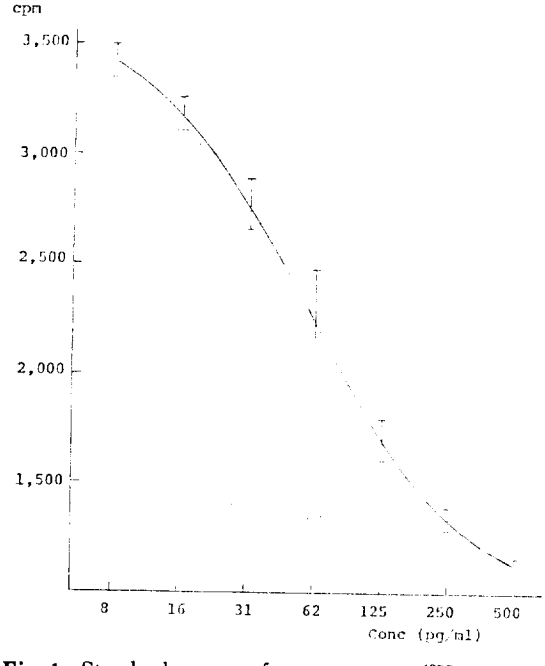

Fig 1. Standard curve of progesterone (^{3}H-progesterone).

sensitivity of the assays was 8 pg per ml. The percentage recoveries of [^{3}H] progesterone was 83.5± 1.6(mean±SD).

The precision of the assay was evaluated by multiple determinations of aliquots from pools of plasma containing different amounts of added progesterone.

The within—and between—assay coefficients of variation were 6.2 and 10.4%, respectively. These results for the determination of progesterone were similar to those in dairy cattle by Choi et al[10] and Gao et al.[11]

The production of progesterone in dairy cattle rises markedly for several days until a plateau of secretion is reached by about day 8 of the cycle. The concentration of progesterone in peripheral blood is low(0.1∼0.8 ng/ml) around the time of estrus.[14,15,16] The concentration of progesterone rises to a mean value of about 5 ng/ml during the luteal phase of the cycle,[17] and a mean peak value of about 6∼7 ng/ml at the end of the luteal phase.[14,18,19] At the end of the estrous cycle, there is a precipitous decline in the blood concentration of progesterone 1∼4 days prior to the onset of estrus.[18,20]

The results of the progesterone determines during the estrous cycle in Korean native cows are shown in Table 1. Plasma progesterone concentration was the lowest at estrus (0.30±0.00 ng/ml, mean± SD), gradually increased to reach a peak (5.04± 1.62) during the luteal phase (Day 8 to 17), gradually decreased thereafter to reach very low concentrations at the next estrus. This patterns were similar to those in plasma progesterone of dairy cows.[12,13]

Several reports[21,22] have dealt with progesterone concentrations in cattle that have become pregnant, compared with their non-pregnant animals in the weeks soon after breeding and have shown the steroid to be a higher level in pregnant animals. In the pregnant cow, progesterone values in peripheral plasma increase with the development of the corpus luteum up to concentrations of the order 5∼10 ng/ml on days 15∼20 after conception; these concentrations remain fairly constant thereafter until shortly before delivery.[23,24] Most investigators[25-27] are now agreed that 20∼23 days after breeding is the most suitable time to make diagnosis of pregnancy.

Table 1. Plasma progesterone concentrations ($\bar{X}$± SD) during the estrous cycle in 10 Korean native cows

Stage in the estrous cycle	Concentration (ng/ml)
Day 1∼ 2	0.30±0.00
2∼ 3	0.38±0.10
3∼ 5	0.89±0.18
5∼ 7	1.73±0.58
8∼17	5.04±1.62
18∼20	1.06±0.63

The low reproductive capacity of the Korean native cows has long been recognized. However, the information on which this was based was in most cases derived from available breeding records, and some work[1,6,7] has been done to further characterize its reproductive capacity. Consequently, the basic endocrinological control of reproduction in this species was poorly understood. To improve reproductive efficiency in the Korean native cows, a better understanding of basic endocrine patterns was required. Hormonal determinations in conjunction with clinical examination have assisted in the determination of normal reproductive patterns and their endocrinological basis. The initiation of the use of the radioimmunoassay techniqe to measure progesterone and estrogen for studying the endocrinology of the estrous cycle inspired the progressive development of immunoassay techniques, in particular progesterone analysis, as a suitable tool for monitoring the reproductive status of the Korean native cows.

Summary

Radioimmunoassay for progesterone in Korean native cows was developed using the anti-4-pregnen-11α-ol-3,20-dione-hemisuccinate bovine serum albumin as an antibody. The final dilution of antibody was 1 : 5,000. The percentage recoveries of [^{3}H] progesterone was 83.5±1.6. The sensitivity of the assay was 8 pg per ml. The intra- and interassay coefficients of variation for the quality controls were 6.2 and 10.4%, respectively. This radioimmunoassay can be applied to puberty, estrus dectection, pregnancy diagnosis, postpartum period and diagnosis of reproductive disorders in domestic animals.

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