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PMSG PROFILES IN SUPEROVULATED AND ANTI-PMSG ANTISERUM TREATED MICE AND HEIFERS WITH ENZYMEIMMUNOASSAY

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A sandwich enzymeimmunoassay (EIA) for pregnant mare serum gonadotropin (PMSG) using a microtiter plate was developed. Sensitivity of the assay to PMSG was 15.6 mIU/ml (0.2 ng/well). The PMSG levels in serum were measured with the EIA in superovulated and anti-PMSG rabbit antiserum treated mice and heifers. In mice, the PMSG blood level was measurable in the serum 4–6 days after intraperitoneal injection of 5–30 IU of PMSG. The administration of anti-PMSG antiserum at the same dose level as PMSG caused a rapid decrease in the PMSG blood level, declining to undetectable levels within 17 hours. In heifers, the PMSG level was measurable at 10–11 days after the injection of 2500 or 3000 IU of PMSG. When antiserum was injected 48 hours after the PMSG injection, the clearance rate of PMSG was affected by the route of the administration. The administration of 3000 units of anti-PMSG antiserum intravenously caused a rapid decline and the disappearance of circulating PMSG within 17 hours. When 3000 units of anti-PMSG antiserum was injected intramuscularly, the PMSG blood level also decreased and became unmeasurable 24 hours after administration; however, it was still detectable for up to 17 hours.

These results indicate that the administration of anti-PMSG antiserum at the proper timing and dosage could lead to successful superovulation through the improvement of hormonal conditions.

Key words: PMSG, anti-PMSG, superovulation, mouse, heifer

INTRODUCTION

PMSG is a glycoprotein containing more than 10% of sialic acid and has a bigger molecular weight (molecular weight 70000) than other gonadotropins. These characteristics allow the induction of superovulation with a single injection. PMSG, however,

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also has the disadvantage of producing embryos of poor quality for the following reasons. PMSG still remains at a certain level in the blood circulation during the luteinizing hormone (LH) phase (McINTOSH et al., 1975 and SCHAMS et al., 1978). It was reported that in PMSG treated animals, the prolongation of ovulation, morphological and functional indisposition in the genital tract, i. e. ovary, oviduct and uterus, was a result of confusion of hormonal control (BOOTH et al., 1975 and BETTERIDGE, 1977). It was also suggested that the indisposition of the genital tract injures the quality of embryos (BOLAND et al., 1978). Anti-PMSG antiserum has been used for the purpose of elimination of circulating PMSG after estrus. BINDON & PIPER (1977) and other investigators reported the improvement of the quality of collected embryos, the ovulation rate and the profiles of various hormones; progesterone, estrogen, follicle stimulating hormone (FSH) and LH after antiserum injection (SAUMANDE, 1980; BOUTERS et al., 1983; SAUMANDE et al., 1984; MOYAERT et al., 1985 and DIELEMAN & BEVERS, 1987). These results suggest that blood PMSG levels decreased after antiserum injection.

Bioassay (COLE & ERWAY, 1941), haemagglutination inhibition assay (ALLEN, 1969) and radioimmunoassay (RIA, FARMER & PAPKOFF, 1979 and MENZER & SCHAMS, 1979) have been established for the assay of PMSG. However, these assay methods have certain disadvantages with respect to cost, sensitivity, measurement time and radiation hazard. It is generally recommended that the EIA technique is a more practical method since it would minimize these problems.

In this study, the EIA for measuring PMSG concentration in serum sample was developed. The PMSG profiles in superovulated and anti-PMSG antiserum treated mice and heifers were measured with the developed EIA.

MATERIALS AND METHODS

Hormone

The PMSG for RIA (UCB-Bioproducts S. A.) was used to make the standard curve. The highly purified PMSG, provided by Dr. H. OKUMURA (3410 IU/mg, Teikoku Zoki Corp.), was used for assay.

Antisera to PMSG and enzyme conjugated antibody

The PMSG antiserum was raised in chickens. Immunization was performed with 9 weekly subcutaneous injections of 3000 IU of 1% formaldehyde treated highly purified PMSG suspended in Freund's complete adjuvant. One ml of the chicken antiserum, purified by affinity chromatography, neutralized 9700 IU of PMSG, as tested by the assay method of SASAMOTO (1962). Rabbit anti-PMSG antiserum provided by Dr. H. OKUMURA (with a neutralizing capacity of 35800 IU/ml, Teikoku Zoki Corp.) and peroxidase labelled affinity purified anti-rabbit IgG goat antibody (Hazelton Biotechnologies Corp.) were used for the assay.

Substrate, buffers and microtiter plate

The 2,2'-azino-di(3-ethyl benzylthiazoline sulfonic acid-6)-diammonium salt (Polyscience Inc.) was prepared by the method of ALBERT et al. (1978).

Sodium phosphate buffer of 0.05 M, pH 8.0, containing 0.5 M NaCl, was used for dilution of the chicken antiserum. Sodium phosphate buffer of 0.05 M, pH 7.2, containing 1% BSA (bovine serum albumin, essentially fatty acid-free, Sigma Chemical Corp.) and 0.05 M NaCl (BSA-buffer) was used throughout the assay procedure. However for washing, sodium phosphate buffer of 0.05 M, pH 7.0, containing 0.05% Tween 20 (Nakarai Chemical Corp.) was used.

A 96 well microtiter plate (Nunc) was used for the assay.

Assay procedure

A 0.2 ml of 1: 500 dilution of chicken antiserum was put to each well and microtiter plate was kept at 4°C for approximately 12 hours. The solution in each well was changed to 0.25 ml of BSA-buffer and kept for 1 hour at room temperature. Washing was done 3 times with 0.25 ml of washing buffer. After this the same procedure was used for washing each time the solution was changed. Each PMSG standard dilution in 0.2 ml of BSA-buffer; 1000, 500, 250, 125, 62.5, 31.3, 15.6 and 7.8 mIU/ml, and the same volume of serum sample were added to each well and incubated for 1 hour at room temperature. The rabbit anti-PMSG antiserum diluted 1: 1000 with 0.2ml of BSA-buffer was added and incubated for 1 hour at room temperature. Peroxidase labelled anti-rabbit IgG goat antibody diluted 1: 2000 with 0.25 ml of BSA-buffer was added and incubated for 1 hour at 4°C. 0.2 ml of substrate solution was added and incubated for 30 minutes until the absorption rate was measured with a spectrophotometer at 405 nm. The PMSG concentration in the serum sample was determined on the standard curve.

Assay precision

Two standard samples of 20 and 500 mIU/ml PMSG, which were prepared with the highly purified PMSG, were used to calculate intra-assay and inter-assay coefficient of variation (CV) as controls for the reproducibility of the EIA. The intra-assay CV was calculated from the mean value of 20 parallel wells in each standard sample. The inter-assay CV was calculated from the mean value of 5 times of operation, using 10 wells in each operation.

Influence of serum to the standard curve

To evaluate the influence of serum to the standard curve, 2 standard curves were made with calf sera of different lots (CS-A, CS-B, Gibco Lab.). Each standard dilution with 2 batches of calf serum was prepared at the same concentration as mentioned above.

Superovulation and blood sampling in mice

A total of 96 immature female ddy mice 28 days old were used. Superovulation was induced with 5, 10 or 30 IU of PMSG (Serotropin, Teikoku Zoki Corp.) and 5 IU of human chorionic gonadotropin (hCG, Gonatropin, Teikoku Zoki Corp.) given 48

hours apart. At the same time as hCG injection, the anti-PMSG rabbit antiserum was injected at the same dose level as for PMSG. In control animals, normal rabbit serum was injected. All injections were prepared in the same volume (0.1 ml) and administered intraperitoneally.

In each group, 2 blood samples were collected by cardiocentesis at 0 (just before PMSG injection), 5, 17, 29, 48, 53, 65, 96 and 144 hours after PMSG injection. Serum obtained from the blood sample was stored at -20°C until assayed.

Superovulation and blood sampling in heifers

A total of 33 heifers, on Day 9–15 of the oestrus cycle, were superovulated with 2500 or 3000 IU of PMSG and 0.5 mg of Prostaglandin $\text{F}_2\alpha$ analogue (Estrumate, I. C. I., $\text{PGF}_2\alpha$) given 48 hours apart. Artificial insemination was performed twice, 55 and 72 hours after $\text{PGF}_2\alpha$ injection. The 3000 units of anti-PMSG rabbit antiserum was injected either intramuscularly or intravenously at 55 and 72 hours after $\text{PGF}_2\alpha$ injection.

In each group, the blood samples were collected 0 (just before PMSG injection), 17, 48 and 96 hours after PMSG injection. In anti-PMSG antiserum treated heifers, blood samples were collected at the time of antiserum injection and followed by 7 or 17 hours post antiserum injection depending on the time when anti-PMSG antiserum was given (55 or 72 hours after $\text{PGF}_2\alpha$ injection, respectively). Twenty four hours after the antiserum injection, another blood sample was taken and then collection was carried out at least every 2 days. Serum obtained from the blood sample was stored at -20°C until assayed.

Statistical analysis

Student's t-test was used to compare the means of the 2 samples.

RESULTS

EIA

An example of the standard curve of developed EIA is shown in Fig. 1. The sensitivity, estimated by the concentration resulting in a response measuring 2 standard deviations away from the zero dose response, was 15.6 mIU/ml (0.2 ng/well). The measurable range of PMSG in 0.2 ml of serum was considered to be between 15.6 mIU/ml (0.2 ng) and 1.0 IU/ml (12.8 ng).

The intra-assay CV was 17.8% (20 mIU/ml) and 9.9% (500 mIU/ml), and the inter-assay CV was 13.0% (20 mIU/ml) and 10.2% (500 mIU/ml), respectively.

The 2 standard curves with 2 batches of calf serum (CS-A and CS-B) and an example of the standard curve with BSA-buffer are shown in Fig. 2. Comparing the 3 standard curves there are no significant differences throughout their effective range.

PMSG profile in mice

In all animals, the PMSG blood level was highest 5 hours after the injection. The mean maximum concentration of PMSG was 563.6 ± 35.1 (group 1), 567.9 ± 22.1 (group 2) and 1250 ± 21.6 mIU/ml (group 3). The PMSG blood level decreased and became unmeasurable within 144 hours (groups 1 and 2, Fig. 3-a and b). However, it still remained at a

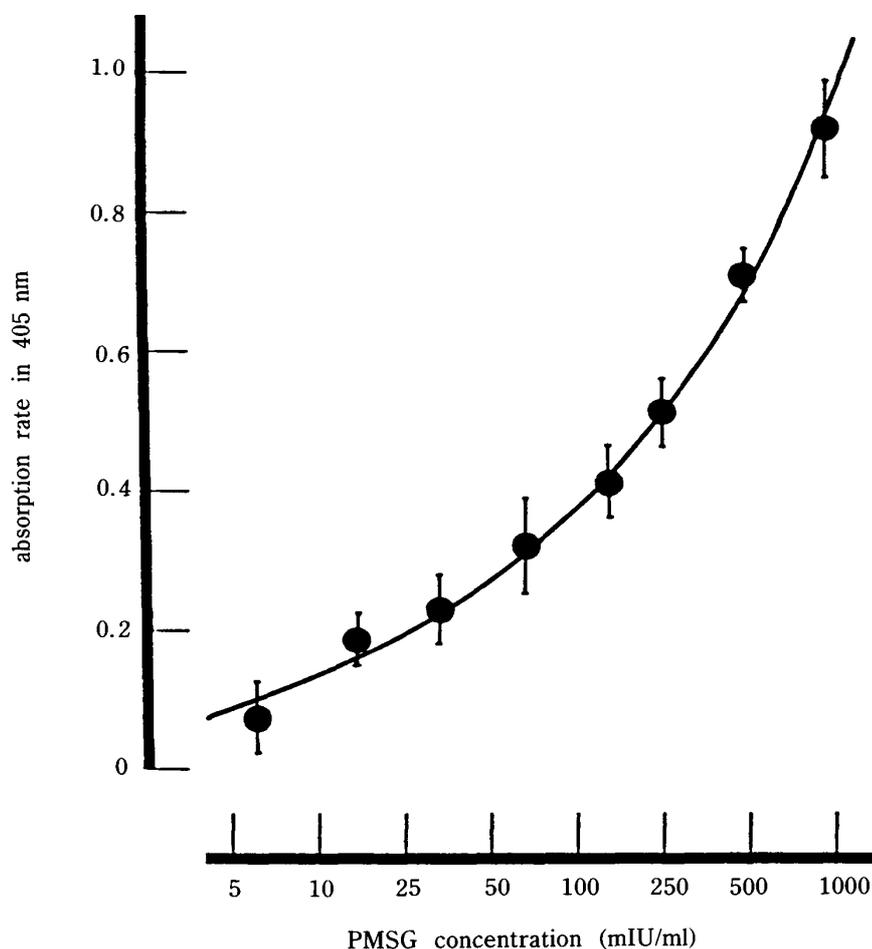


Fig.1 Standard curve for EIA of PMSG. Each point represents mean \pm SD from 5 replicate assays.

measurable level until 144 hours (group 3, Fig. 3-c).

In animals of all experimental groups, the PMSG blood levels were still measurable 5 hours after antiserum injection, however, they were significantly lower than that of the control group ($P < 0.01$). PMSG blood levels became unmeasurable 17 hours after antiserum injection (Fig. 3-a, b and c).

PMSG profile in heifers

In all animals, PMSG blood level was highest 7-17 hours after the injection. The mean maximum concentration of PMSG was 101.0 ± 9.6 ($n=12$) and 110.6 ± 19.7 ($n=8$) mIU/ml when 2500 and 3000 IU of PMSG were injected, respectively. The PMSG blood level was measurable 10-11 days after the injection. The PMSG profile in a heifer injected 3000 IU

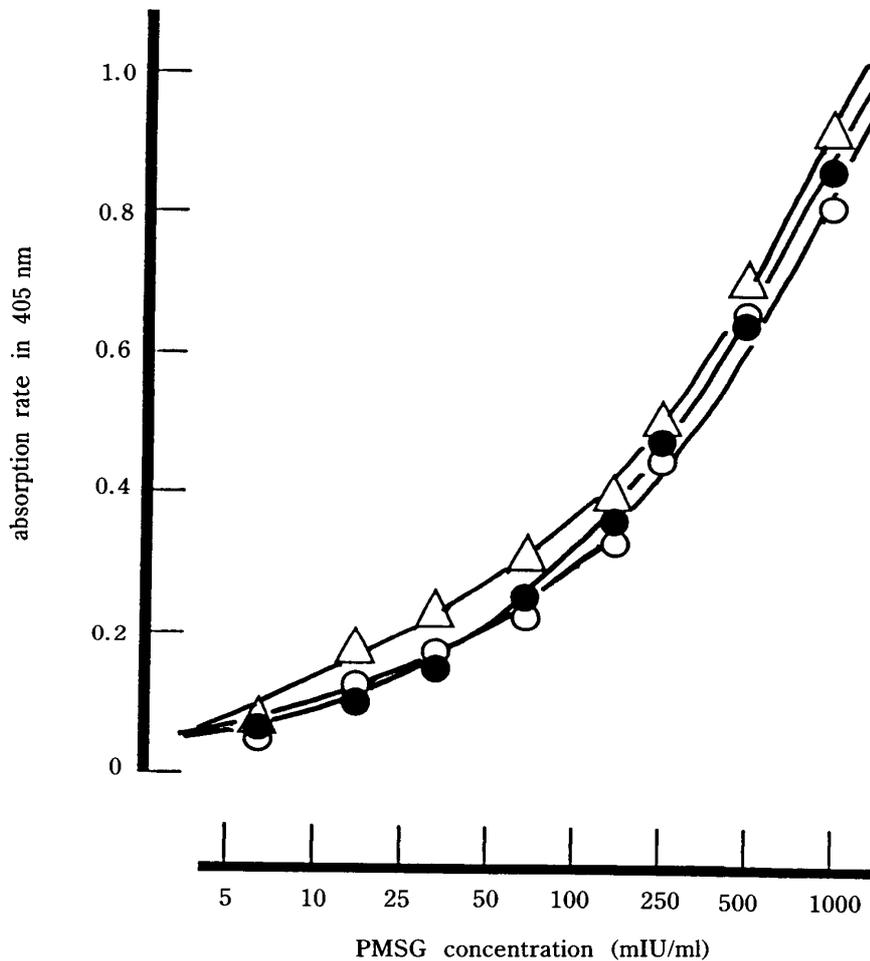


Fig.2 Influence of calf serum (CS) to standard curve.

- : standard curve with BSA-buffer
- : standard curve with CS-A
- △—△: standard curve with CS-B

of PMSG was shown in Fig. 4-a. In all animals which were given anti-PMSG antiserum, a rapid decline in blood PMSG level was recorded. When the antiserum was injected intravenously, the PMSG blood level became unmeasurable within 7-17 hours. When the antiserum was injected intramuscularly, PMSG blood level was still measurable 7-17 hours after the injection, but, it was significantly lower than that of control group ($P < 0.01$). The PMSG blood level became unmeasurable within 24 hours after antiserum injection. The PMSG profile in a heifer of each treatment group was shown in Fig. 4-b and c.

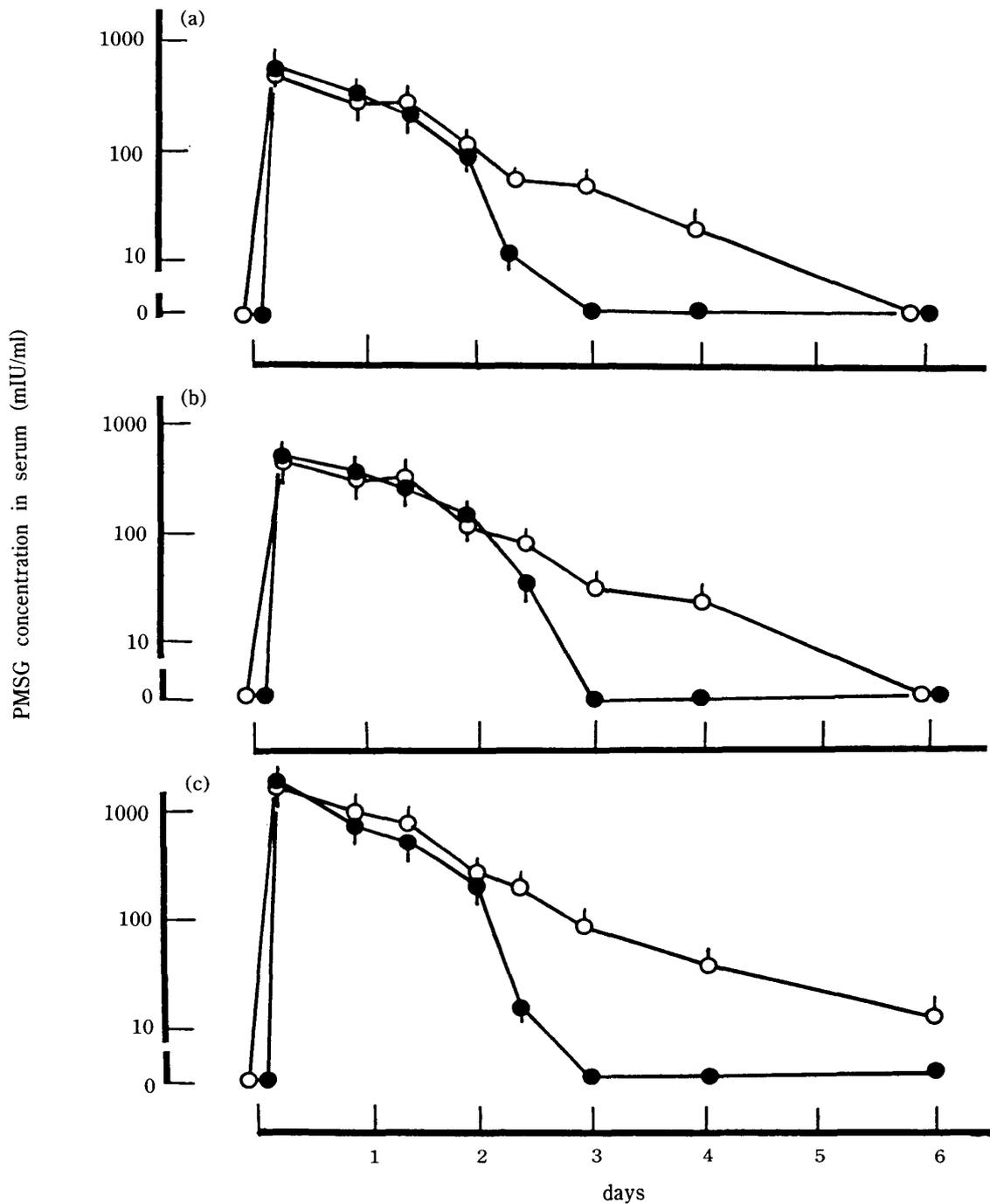


Fig.3 PMSG profiles in superovulated mice. Control animals (—○—) were given 5 (group 1, a), 10 (group 2, b) or 30 (group 3, c) IU of PMSG and 5 IU of hCG at 48 hours apart. Experimental animals (—●—) were given anti-PMSG antiserum at the same dose level as PMSG at the time of hCG injection. Each point represents mean \pm SD from 2 blood samples.

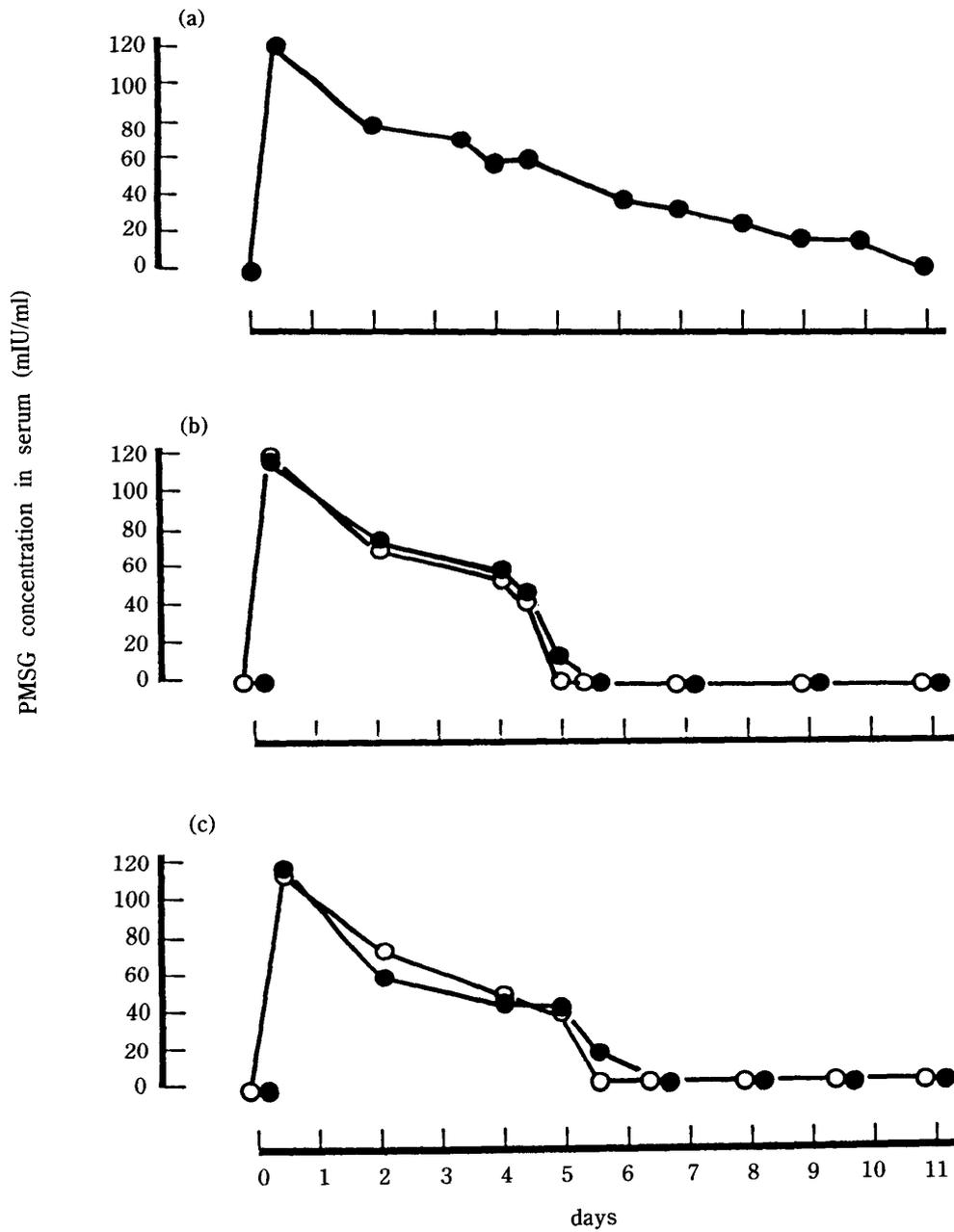


Fig.4 PMSG profiles in superovulated heifers. Control animal was given 3000 IU of PMSG and 0.5 mg $\text{PGF}_2\alpha$ at 48 hours apart (a). Experimental animals were given anti-PMSG antiserum intravenously (—○—) or (—●—) at 55 (b) or 72 (c) hours after $\text{PGF}_2\alpha$ injection.

DISCUSSION

In this study, good reproducibility of the sample measurement was observed and there was no significant difference between the standard curve of BSA-buffer and those of calf serum. These results demonstrate that the developed EIA is suitable for measuring PMSG concentration in serum samples. The sensitivity of the EIA (0.2 ng) is slightly lower than that of RIA; 10–50 pg (FARMER & PAPKOFF, 1979) and 0.1 ng (MENZER & SCHAMES, 1979), however, sensitivity is sufficient for practical use. In other assay methods; RIA, bioassay and haemagglutination inhibition assay, the assay procedures require more than 24 hours. On the contrary, the assay procedure of this EIA can be done within 5 hours with the prepared microtiter plate. The chicken antiserum and BSA-buffer treated microtiter plate can be stored at -40°C without significant difference in the result between assay with the stored microtiter plate and normal assay procedure (data not shown). In addition, this EIA using a 96 well microtiter plate allows the treatment of many serum samples at a time.

The study showed the long life of injected PMSG (5–30 IU) in the mouse (4–6 days). It has been reported that the biological life of small concentrations of PMSG (2–3 IU) in immature mouse was 54–60 hours (SASAMOTO et al., 1972) and 6 days (LADMAN, 1964). The age of mouse and dosage of PMSG used in the present experiment were different from those of the previous reports. But the dose of PMSG per unit body weight (0.18–0.36 IU/gBW, groups 1, 2) was similar to that of the previous report (0.32–0.43 IU/gBW, SASAMOTO et al., 1972).

In mice of group 3, a large dose of PMSG per unit body weight (1.1 IU/gBW) was injected. It was reported that the profile of a large dose of PMSG given in sheep (McINTOSH et al., 1975) and cows (SCHAMS et al., 1978) showed a double exponential model. The profile of PMSG in mice of group 3 was, however, that of an exponential model. In our experiment, the interval of blood collection was too wide (5–17 hours) to clearly show the profile of PMSG immediately after the injection as compared with that of the previous reports.

This study confirmed a rapid decline of PMSG blood level in all anti-PMSG antiserum treated mice. The blood PMSG concentration was still at a measurable level (12.5–28.7 mIU/ml) 5 hours after antiserum injection. Since these values are at a biologically effective level (SASAMOTO, 1962), anti-PMSG antiserum seems to need to be administered earlier to eliminate effects on the embryo.

The PMSG profile in the cow after 1500–3000 IU of PMSG injection has been reported with RIA technique (SCHAMS et al., 1978). In this report, the PMSG blood level was highest 24 hours after the injection, approximately 80 and 100 mIU/ml when 1500 and 3000 IU of PMSG, respectively, were injected. Also, the PMSG blood level was detectable 10 days after the injection. The PMSG profile in our experiment is in agreement with that of the previous report with regard to the maximum concentration and the duration from PMSG

injection to its disappearance from the circulation. However, the time of the highest PMSG blood level was different. In our study, the highest PMSG blood level was obtained 7–17 hours after PMSG injection.

The PMSG blood level decreased rapidly after antiserum injection in all heifers. This result agrees with the PMSG profile in heifers (DIELEMAN & BEVERS, 1987), who reported that the pattern of the PMSG concentration showed a marked decrease of about 85% within 1 hour of intravenous administration of the monoclonal anti-PMSG antibody. In the present study, the clearance rate of circulating PMSG was affected by the route of administration of antiserum. 3000 units of anti-PMSG antiserum injected intravenously neutralized circulating PMSG more rapidly than when it was injected intramuscularly.

This study showed that the PMSG injected to induce superovulation disappeared within several hours after antiserum administration both in mice and heifers. Further studies on the proper timing of antiserum injection, the relation between the disappearance of circulating PMSG and its biological effect on embryos via the genital tract; ovary, oviduct and uterus, should be carried out.

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