DETERMINATION OF PORCINE PLASMA FOLLITROPIN LEVELS DURING SUPEROVULATION TREATMENT IN COWS.

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Received for publication : *August 17, 1987*
Accepted : *May 18, 1988*

ABSTRACT

Porcine follicle stimulating hormone (pFSH) and porcine luteinizing hormone (pLH), are widely used to induce superovulation in cows. An advantage of this treatment is that the LH:FSH ratio can be varied to optimize the growth of the ovarian follicles. However, due to the relatively short half-life of FSH, the superovulatory treatment requires numerous injections.

A performant radioimmunoassay system (sensitivity=0.2 ng/ml plasma) was used to determine plasma pFSH levels in cows that were superovulated with 2 daily injections of 4 Armour Units (A.U.) of pFSH for 4 d. From plasma profiles, the half-life and the disappearance of pFSH were estimated at 5 h and at 10 to 12 h, respectively, confirming the necessity of using two daily injections.

Key words: plasma pFSH level, superovulation, cow

Acknowledgement:
This research was supported by IRSIA Institute, Rue de Crayer, 6 B.1050 Bruxelles. We thank the NIH for the gift of hormones.

INTRODUCTION

Superovulation is used to produce several oocytes that can be fertilized with a single insemination and to furnish many embryos of good quality.

The most commonly used superovulatory agents in the cow are pregnant mare serum gonadotropin (PMSG) and porcine follicle stimulating hormone (pFSH). These hormones are glycoproteins. These glycoproteic hormones are constituted of two polypeptidic chains (α and β subunits) on which sugars and sialic acid are grafted. These characteristics explain the protection of these hormones from hepatic degradation and renal filtration. Due to its high content of sialic acid, PMSG has a very long half-life (1). Significant PMSG levels were still detected in blood plasma 10 d. after an intramuscular injection (2). This long half-life can produce adverse effects on superovulatory responses since high plasma PMSG levels for more than 5 to 6 d. stimulate ovarian follicles that produce high levels of estrogens after estrus following superovulation. To minimize this effect, the injection of an antiserum against PMSG 5 d. after initiation of treatment has been proposed (3). The PMSG-antiPMSG treatment resulted in better superovulatory responses, including higher ovulation rate, decreased number of unruptured follicles and decreased number of cysts. Subsequently, other authors adopted this treatment (4, 5), and recently, the utilization of a monoclonal antibody has been proposed (6, 7).

Porcine pituitary extracts rich in FSH can also be used for superovulation. In fact, pFSH is the substance most commonly used to induce superovulation in cows. Moreover, recent studies point out the importance of an adequate LH:FSH ratio to increase the quality of embryos produced (8, 9).

The superovulation treatment consists of administrating two daily doses pFSH varying from 2 to 6 Armour Units (A U) injected over a 4-d period (the total pFSH amount for the treatment: 28 to 40 A.U.; 10-13). This treatment can also be carried out at constant and at decreasing unitary doses (14). Actually, the latter procedure provides the best superovulatory responses in cattle. On the other hand, the use of two daily injections is a technical inconvenience, and it can be the origin of errors in dose and injection time. It could also constitute a source of stress for some of the treated animals.

The object of our study was to determine plasma pFSH levels in cows superovulated by repeated injections of porcine pituitary extract. A radioimmunoassay for pFSH was used to detect small plasma pFSH concentrations.

MATERIALS AND METHODS

Porcine FSH and porcine LH were purified according to the methods of Closset et al. (15) and Closset and Hennen (16). The highly purified FSH had a specific activity 100 times greater than pFSH-NIH-P_1 as measured by radioreceptorassay.

For the production of antiserum, two rabbits were immunized against porcine FSH according to the method of Vaitukaitis et al. (17). The more sensitive antiserum was employed at a final dilution of 1:20,000.

The purified pFSH hormone was iodinated with ^{125}I by the enzymatic procedure of Thorell and Johansson (18). The separation of labeled protein from free iodine was achieved by gel filtration through a Sephadex G-75 column. The labeled hormones were stored at -20°C. Before use, the tracer was passed through a Sephadex G-75 column to remove the damaged hormone.

Dilutions and incubations were performed in 0.025 M TRIS-HCl; pH-7.6 containing 0.1 % (w:v) bovine serum albumin and 0.01 % (w:v) neomycine sulfate. Plasma (200 µl), TRIS buffer (100 µl) and antiserum (100 µl) were incubated for 24 h at 4°C. Then 100 µl of tracer solution (20,000 cpm per tube) was added. The tubes were incubated for 24 h. at 4°C, then a second antibody coupled to cellulose (DASP) was added. The addition of DASP (1 ml) was followed by incubation for 24 h. at 4°C. The tubes were centrifuged and the supernatant was aspirated. The pellet was then washed with TRIS buffer (3 ml). After centrifugation, the supernatant was aspirated and radioactivity was counted. In order to neutralize the interference of plasma, 200 µl of plasma from untreated cows were added to the standard curve. Results are expressed in terms of percentage of B/B_0 where B represents the bound counts/minute in the presence of unlabeled antigen, and B_0 is the radioactivity bound to antibody in the absence of unlabeled antigen.

Two Holstein Frisian cows (A and B) were superovulated with pFSH injections: 32 mg of pFSH administered over 4 d in eight equal doses at 12-h intervals. Blood samples were collected during the day at 1, 3, 5, 9 and 12 h and during the night at 1, 3, 5 and 12 h after each injection.

All blood samples were collected by jugular puncture in heparinized tubes. The blood was centrifuged for 20 min at 4°C and 2,500 g. The plasma was frozen at -20°C and stored until assay.

RESULTS

At a final dilution of 1: 6,000, the selected antiserum bound 91% of the ^{125}I radiolabeled porcine FSH. At the working dilution (1:20,000), 40 ± 5% of the tracer was bound.

Figure 1 shows the standard curve for pFSH. Bovine LH did not produce any significant displacement of the tracer and bovine FSH only slightly inhibited the binding of the tracer. The sensitivity of the system corresponds to 0.2 ng pFSH/ml plasma.

The pFSH profiles are given in Figure 2. The pFSH concentration increased immediately after i.m. injection and reached a maximum (mean value of the peaks: 0.51 ng/ml) 3 h later. Then it uniformly decreased and the pFSH could not be detected 12 h after the injection. From these data, the half-life of pFSH in the cow was estimated to be approximatively 5 h.

DISCUSSION

In comparison with other described radioimmunoassays for the measurement of porcine FSH, our system is highly specific and more sensitive. Its sensitivity was 0.2 ng/ml plasma compared with 2.5 ng/ml and 0.5 ng/ml in other studies (19, 20). The use of a 24-h preincubation period probably accounts for this increased sensitivity.

The development of a sensitive radioimmunoassay for pFSH allowed us to determine the plasma pFSH profiles induced by superovulatory treatment. To our knowledge, these profiles have never been previously described.

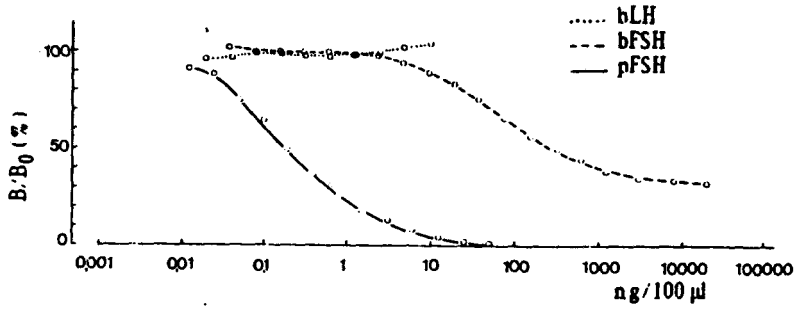

Figure 1. Radioimmunoassay of pFSH: inhibition curves of pFSH, bFSH and bLH.

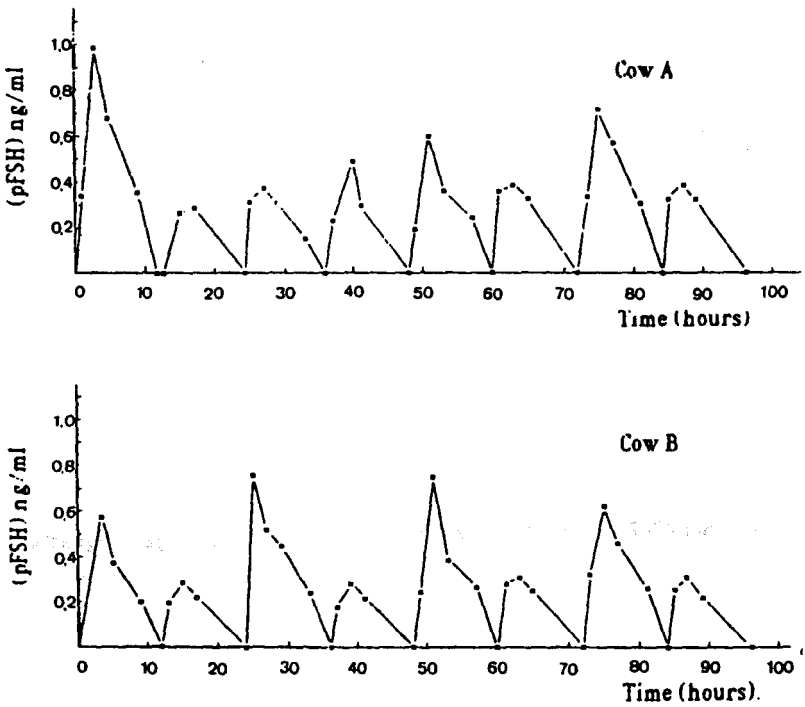

Figure 2. Plasma pFSH levels in Cows A and B superovulated by repeated injections.

In our study and elsewhere (21), the commonly administered doses of 4 AU pFSH/injection were utilized and produced good superovulatory responses. The maximum plasma pFSH concentration after each injection was approximatively 0.51 ng/ml. Since a 4 AU dose of pFSH is equivalent to 55 µg of pure hormone, and since extracellular fluid volume for cows is approximately 90 l (one-fifth of the weight), the theoretically calculated concentration of pFSH after injection is similar to the experimentally determined value. Because the experimental data for each injection were limited, it was not possible to calculate mathematically the half-life of pFSH. However, it was estimated to be approximatively 5 h; this is similar to the value reported in the literature for ovine FSH in cattle ($t_{1/2}$ oFSH: 301 ± 23 min; 22).

The plasma profiles clearly confirm the necessity for two daily injections of pituitary extracts containing pFSH and pLH, for 4 d. to obtain effective stimulation of ovaries. However, this regimen is inconvenient to administer, and the development of a controlled release system, which would release pFSH over a 4-d. period, would be a major advance. The pFSH sensitive radioimmunoassay described in this study will be used to investigate the release of pFSH in vivo using a biodegradable implant.

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