Are antibodies responsible for a decreased superovulatory response in goats which have been treated repeatedly with porcine follicle-stimulating hormone?

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ARE ANTIBODIES RESPONSIBLE FOR A DECREASED SUPEROVULATORY RESPONSE IN GOATS WHICH HAVE BEEN TREATED REPEATEDLY WITH PORCINE FOLLICLE-STIMULATING HORMONE ?

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ABSTRACT

Repeated administration of xenogenic gonadotropins in human or animal species may be responsible for antibody production and refractoriness. An experiment was conducted in which goats were treated with porcine FSH (p-FSH) at 6-week intervals for a period of 7 months. A sensitive radioimmunoassay (RIA) was used to detect antibodies to p-FSH in plasma samples taken at short-term intervals during a 7-month period. Antibodies appeared after the first injection, and levels increased following booster injections. A high correlation rate existed between antibody level and superovulatory response. Refractoriness in goats was associated with a high level of antibodies.

Key words: superovulation, gonadotropins, antibodies, goats, refractoriness

INTRODUCTION

Superovulation followed by the recovery of embryos and transfer to appropriately synchronized recipients has proved to be an effective means of increasing the contribution of superior females to the gene pool of various animal species (1). This technique has recently been applied to the genetic improvement of goats of different breeds, including the Angora and Saanen. However, the low number of transferable embryos recovered per

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superovulated female still constitues a major limitation of the technique. According to Amstrong (2), superovulation with porcine pituitary extracts yields more and better quality embryos than with pregnant mare serum gonadotropin (PMSG); after transfer to recipients, superovulation induced by pituitary extracts produced 7.5 ± 0.6 (mean ± SEM) newborn goats (kids) per donor versus 4.8 ± 0.6 when PMSG was used.

Along with better knowledge of superovulation methods and drugs, embryo recovery rates have recently improved for the ovine and caprine species. In the early experiments, embryos were collected surgically with the commonly observed complication of adhesions. Goats were often eliminated after two or three surgeries. With the advent of laparoscopy, it has become possible to repeatedly collect embryos in the same female (3,4).

However, when p-FSH was used to repeatedly superovulate goats, the mean embryo recovery rate decreased sharply after two or three treatments (4,5). Similar observations have been reported by Nuti et al.(6) for goats superovulated with porcine pituitary extracts rich in p-FSH as well as by other authors, who induced superovulation with PMSG in the cow (7,8,9), ewe (10,11), rat (12), rabbit (13) and monkey (14).

In goats treated repeatedly with p-FSH, three different hypotheses have been suggested to explain the decrease in the number of ovulations (corpora lutea) and embryos recovered. First, this decrease could be due to seasonal effects, as when superovulation is initiated during the breeding season and is continued into the period of anestrus. Second, the follicular population in the ovary may be adversely affected by repeated superovulation. Third, a progressive resistance to gonadotropins may occur in animals which have been repeatedly injected with xenogenic proteins. To explain the decrease in the response to superovulation in caprine species, we developed a highly sensitive technique using ^{125}I-pFSH to measure antibodies to p-FSH in goat plasma or serum (5).

To investigate the antibody response (if any) to exogenous p-FSH, we collected blood samples at regular intervals from goats that were repeatedly injected with p-FSH for superovulation induction. These blood samples were screened for p-FSH-binding activity, and the results were correlated with the superovulatory response.

MATERIALS AND METHODS

Animals and Superovulation Induction

Twelve adult goats (six Alpines and six Saanens) were superovulated five times at 47-day intervals beginning in October. Synchronization or induction of estrus was achieved by injecting 100 µg of cloprostenol (a $PGF_{2\alpha}$ analogue, Estrumate, Coopers, Meaux, France) on the ninth day of an 11-day progestagen treatment (regimen: vaginal sponge impregnated with 45 mg of fluorogeston acetate, Intervet, Angers, France). To induce superovulation, 16 Armour Units of p-FSH prepared by Combarnous (INRA, France) were administered in six decreasing doses (4, 4, 2, 2, 2, 2 A.U.), injected at 12-hour intervals during the last three days of the progestagen treatment.

The superovulatory response was assessed by the number of corpora lutea counted by laparoscopy 6 to 7 days after the onset of estrus.

Blood samples (10 ml) were collected from all animals just before the first injection and again at 19 days after the last injection of FSH in each superovulatory treatment. However, after the fourth and the fifth treatments, a series of seven blood samples were collected at Days 7, 14, 19 and 47 after the last FSH injection in order to establish an antibody titer curve for each animal.

Purified Porcine Follicle-Stimulating Hormone (p-FSH)

Porcine FSH was purified from pituitary glands according to the method of Closset and Hennen (15). As determined by the Steelman-Pohley bioassay (16), the activity of the final purified preparation was 98 ± 27 (mean ± SD) times higher than that of the NIH preparation P1.

Radiolabeling With ^{125}Iodine

The purified p-FSH hormone was radioiodinated with ^{125}I by the enzymatic procedure of Thorell and Johansson (17). Immediately after the reaction (4.5 minutes), the radiolabeled hormone was separated from free ^{125}Iodine by chromatography on a Sephadex (Pharmacia) -75 column (0.9 x 30 cm) equilibrated with Tris HCl 0.01 M, pH 7.6 containing 0.1 % bovine serum albumin (bSA).

Antibody Detection in Plasma Samples.

All dilutions of serum or tracer were performed in Tris-HCl buffer 0.025 M, pH 7.6 containing bSA 0.1 % and neomycine sulfate 0.01 %. The incubation volume was always

500 µl. Reagents were added in the following order: 300 µl of buffer, 100 µl of the 1/10 diluted serum, 100 µl of ^{125}I-pFSH (20,000 cpm). This amount corresponds to 255 pg of radiolabeled FSH.

Incubation was carried out for 16 hours at 20°C, following which 100 µl of the undiluted donkey anti-goat immunoglobulins were added. Incubation was carried out for 1 additional hour at 20°C. Then 500 µl of 4% (w/v) polyethylene glycol (PEG MW 10,000 Merck Inc., Darmstadt, F.R. Germany) diluted in water were added. The tubes were centrifuged at 2,500 g for 20 minutes, and the supernatants were aspirated carefully.

The pellets were washed with 3 ml of Tris bSA buffer and were centrifuged. After aspiration of the supernatants, the pellets were counted for radioactivity in a gamma counter with a 60% efficiency for ^{125}I. Nonspecific binding (NSB) of ^{125}I p-FSH was determined in all experiments using plasma (in duplicate) obtained from 10 young female goats which had never been treated with exogenous gonadotropins. Only binding above NSB + 2 C.V. (NSB + 0.52%) was considered significant.

Evaluation of Results

The data are presented in the text as superovulatory responses (mean ± SEM): the number of corpora lutea counted by laparoscopy for each p-FSH treatment. The same data are presented graphically as the mean superovulatory response, with the minimum and maximum response for each treatment. The specific binding percentages (mean + SEM) of ^{125}I-pFSH to plasma are also presented for each treatment in the longitudinal study of repeated induced superovulations.

Analysis of variance based on a 2 ways classification was used to assess significance of differences between means of superovulatory responses and binding percentages.

Correlations were calculated between superovulatory responses and antibody levels measured just before and after treatment (7 and 14 days after the first day of p-FSH administration).

RESULTS

A sensitive and specific radiometric assay allowed antibodies measurement in serum taken from 12 adult goats treated at 47-day intervals with a 16 Armour Unit dose of p-FSH to induce superovulation.

As determined by the selfdisplacement method of Roulston (18), the specific radioactivity of the tracer was 78,330 cpm/ng or 1.956 µCi/picomole. Significant binding

(0.52% above the NSB) corresponds to 0.414 femtomoles of FSH bound per 1 ml of undiluted plasma.

Superovulatory responses were high on the first induction (13.7 ± 2.1 C.L., mean ± SEM) and on the second treatment (15.2 ± 2.05 C.L.). Thereafter, the number of ovulations decreased significantly ($P < 0.01$) to 9.8 ± 2.48, 9.1 ± 2.09, and 7.4 ± 2.35 corpora lutea at the third, fourth and fifth inductions, respectively (Figure 1).

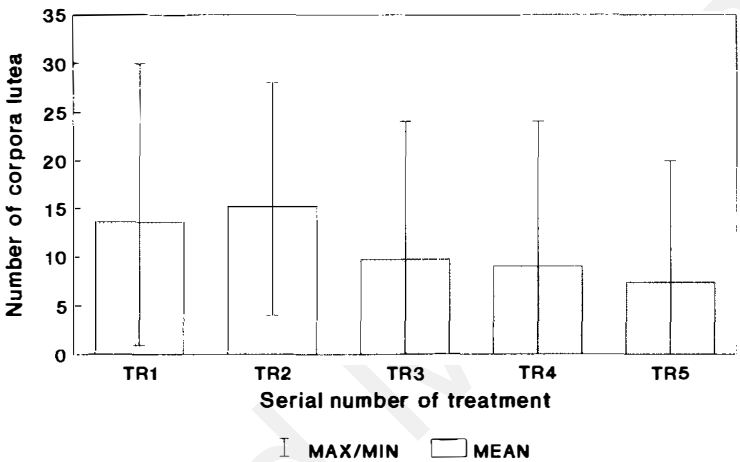

Figure 1. The ovulation rate in goats following repeated superovulation with p-FSH.

When measured 33 days after the first treatment and 19 days after the other superovulatory treatments, binding of ^{125}I-pFSH was 0.98 ± 0.34 (mean ± SEM), 3.96 ± 0.81, 5.69 ± 1.50, 12.47 ± 3.44 and 17.75 ± 5.81 %, respectively (Figure 2).

The series of plasma samples collected after the fourth and fifth p-FSH administrations allowed us to measure the profiles of the antigenic responses (Figure 3).

From the correlations study, it appeared that the decrease in mean superovulatory response was highly and negatively correlated with binding levels 19 days after the last injection of p-FSH ($r = -0.495$; $n = 48$; $P < 0.01$). Negative correlations were also observed between binding

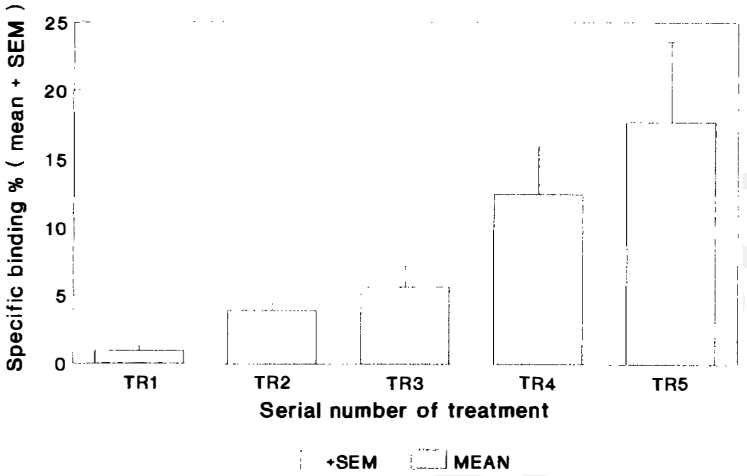

Figure 2. The p-FSH binding levels of plasma taken from goats repeatedly superovulated with p-FSH.

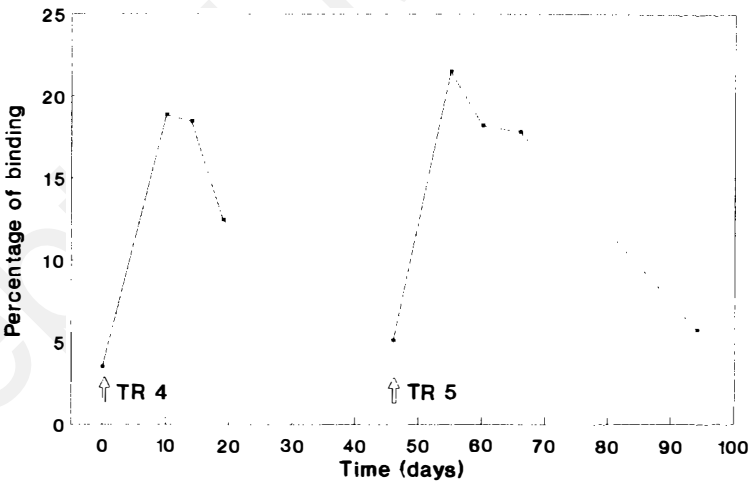

Figure 3. Immune humoral response to p-FSH following the fourth and fifth treatments.

levels measured 28 days before the next treatment ($r = -0.44$; $n = 36$; $P < 0.01$). However the best correlations were obtained for blood samples collected 14 days after the last p-FSH injection (Table 1).

Table 1. Correlation between the number of corpora lutea at fourth and fifth treatments and the anti-pFSH antibody levels after the fourth treatment.

Correlation (r)	Anti-pFSH antibody level at Day 0[a]	Day 10	Day 14	Day 19	Day 47[a]
CL at fourth treat.	-0.486	-0.672*	-0.707*	-0.643*	-0.497
CL at fifth treat.	NC[b]	-0.492	-0.579*	-0.512	-0.411

* $P < 0.05$.
[a] Days 0 and 47 correspond to the initiation of the fourth and fifth treatments.
[b] Not calculated.

DISCUSSION

Immunization of patients or animals to xenogenic polypeptide, protein or glycoprotein hormones is well known. Yalow and Berson (19,20) developed their first radioimmunoassays for insulin using the sera of patients resistant to insulin as a source of antibodies to bind the radioactive tracer. In 1953, Willett et al. (7) described the refractoriness of cows repeatedly superovulated with gonadotropins, but these authors were unable to show circulating antibodies.

Thereafter, using a bioassay method, Jainudeen et al.(8) showed that antigonadotropins in the serum of cows resistant to PMSG inhibited the follicular stimulating properties of the hormone (PMSG). However, they did not observe adverse effects on follicular development and on ovulation resulting from endogenously secreted gonadotropins (FSH and LH). In that paper, Jainudeen et al. (8) were unable to demonstrate the existence of anti-PMSG antibodies by the Ouchterlony immuno diffusion method probably due to the lack of sensivity of the technique.

To investigate the potential antigenic effects of xenogenic gonadotropins in ruminants (cows, ewes and goats), we decided to develop a radiometric measurement of conjectural antibodies in the plasma. In cows injected with porcine FSH to induce superovulation, we have never detected significant binding of the tracer; more than 100 cows were investigated, many after 2 to 10 superovulatory treatments. The lack of immunogenicity of p-FSH in the

bovine species could be explained by the relatively high homology of p-FSH molecules and bovine FSH (b-FSH) molecules. In fact, the amino acid (aa) sequences of b-FSH (21) and p-FSH (22) only differ in 4 aa for the α subunit and 9 aa for the ß subunit.

In contrast, in goats we have detected a marked immunological response to p-FSH. Superovulatory responses rapidly decreased when gonadotropins were injected repeatedly. Moreover antibodies were easily detected in the plasma after one treatment and the levels increased according to the serial number of administrations. High correlation rates existed between the lack of response to superovulatory treatment and the anti-pFSH immunoglobulin concentration in the plasma.

The binding levels of ^{125}I-pFSH reliably reflect antibody production and disappearance. The rate of anti-pFSH antibody appearence corresponds to the curve characteristic of immuno humoral secondary reaction: a short latency period, a maximal level period (Days 14 and 19) and a relatively long decreasing phase (23).

We did not attempt to measure the increasing levels of antibodies during the latency period because our radiometric method was unable to determine reliably antibody concentration in the presence of hormone. We began the first measurement 7 days after the last FSH injection, when circulating levels of p-FSH were very low. The half-life of p-FSH in cows was determined as 5 hours and disappearance was estimated to take place at 10 to 12 hours (24).

In our study, negative correlations were observed between the ovulation rate and binding levels at 7, 14 and 19 days after treatment. In addition, negative correlations were also observed between binding levels measured 28 days before the next treatment and the ovulation rate of this next treatment ($r = -0.444$; $n = 36$; $P < 0.01$). In this study with repeated superovulation induction in goats, the individual level of p-FSH binding 19 days after treatment can constitute a good predictive test for the next superovulatory response in this animal. The use of such correlations to predict superovulatory response could be used to facilitate the detection and elimination of non responding (immunized) females from ongoing superovulation programs.

In conclusion, our study demonstrates that when goats are immunized against p-FSH during superovulatory treatments, the commonly observed decrease in superovulatory response with repeated treatments may be explained by antibodies neutralizing the exogenous gonadotropins.

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