THE EFFECT OF IMMUNIZATION AGAINST INHIBIN ON THE MEDIUM AND LARGE FOLLICLES IN DAIRY HEIFERS[1]

PENGARUH IMUNISASI INHIBIN TERHADAP FOLIKEL BERUKURAN SEDANG DAN BESAR PADA SAPI PERAH

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ABSTRACT

The study was conducted to investigate the effect of either immunization against recombinant ovine inhibin (r-oINH-a3) or stimulation with PMSG on the number of follicles available for aspiration in dairy heifers. Twenty four Friesian heifers were randomly allocated to 3 treatment groups; placebo (C, n=7), PMSG (P, 750 IU per week during the 6 week experimental period; n=7) and inhibin immunization (I, 250 μg 4 weeks prior to and 250 μg at the start of the experimental period; n=10). During the experimental period, the total number of follicles =2 mm was not affected by treatment (C=17.0, P=16.0, I=17.6, P>0.05) but the inhibin immunization increased the number of follicles between 5-9 mm (C=4.2, P=4.1, I=7.1, P<0.001) and =10 mm (C=1.1, P=1.4, I=4.4, P<0.001) which enabled more follicles to be aspirated from the I heifers (C=7.9, P=7.4, I=12.6, P<0.001). As a result, there were fewer follicles 2-4 mm on the ovaries of I heifers (C=11.6, P=10.5, I=6.1, P<0.005). Immunization against inhibin increased the number of medium (5-9 mm) and large (=10 mm) follicles through an increased rate of development of follicles =2 mm.

Key Words : inhibin, follicles, immunization, dairy heifers

ABSTRAK

Penelitian ini dilakukan untuk mengetahui pengaruh imunisasi rekombinan inhibin

[1] Paper was presented at The National Scientific Meeting in Veterinary Sciences, Balitvet Bogor, 12-13 March 1996.

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(r-oINH-a3) maupun pemberian preparat PMSG terhadap jumlah folikel yang dapat digunakan untuk program aspirasi folikel pada sapi perah. Sebanyak 24 ekor sapi FH dibagi dalam 3 kelompok perlakuan; kelompok plasebo (C, n=7), PMSG (P, n=7, mendapat suntikan 750 IU PMSG setiap minggu selama 6 minggu periode penelitian) dan inhibin (I, n=10, diimunisasi dengan 250 µg inhibin pada 4 minggu menjelang dan diulang kembali dengan dosis yang sama pada saat penelitian dimulai). Selama periode penelitian, jumlah total folikel yang berukuran =2 mm tidak dipengaruhi oleh perlakuan yang diberikan (C=17,0, P=16,0, I=17,6, P>0,05) akan tetapi imunisasi inhibin menyebabkan terjadinya peningkatan jumlah folikel berukuran 5-9 mm (C=4,2, P=4,1, I=7,1, P<0,001) dan =10 mm (C=1,1, P=1,4, I=4,4, P<0,001). Hal ini memungkinkan lebih banyak folikel yang dapat diaspirasi dari sapi kelompok I (C=7,9, P=7,4, I=12,6, P<0,001). Jumlah folikel berukuran 2-4 mm pada ovarium kelompok I menjadi lebih sedikit (C=11,6, P=10,5, I=6,1, P<0,005). Imunisasi inhibin meningkatkan jumlah folikel berukuran menengah (5-9 mm) dan besar (=10 mm) melalui peningkatan pertumbuhan folikel berukuran kecil, =2 mm.

Kata-kata Kunci : inhibin, folikel, immunisasi, sapi perah

INTRODUCTION

Inhibin is a dimeric protein hormone composed of two dissimilar, disulfide-linked subunits [termed α and ß, with an apparent molecular weight of 58 kd, which dissociates in 43 kd and 15 kd] and is involved in the negative feedback regulation of gonadotrophin secretion, preferentially follicle-stimulating hormone (FSH) (Burger and Iragashi, 1988). An inverse relationship between concentrations of FSH and inhibin in the circulation was observed in cows during the follicular phase and the early luteal phase in the oestrous cycle (Taya *et al.*, 1991), and was also observed in cows after treatment with equine chorionic gonadotrophin (eCG) to induce superovulation (Kaneko *et al*, 1992). These earlier reports suggest that inhibin is a possible regulator in the control of FSH secretion in cows.

In accordance with the prevailing view that follicular development is controlled by complex local and systemic feedback mechanisms involving gonadotrophins from the pituitary gland and steroids and proteins from the ovaries, manipulation of either endogenous gonadotrophins or local regulation by immunization against inhibin may increase follicles number and perhaps the quality of the oocytes. The present study was conducted to test this hypothesis, that immunization against inhibin would increase follicular development in heifers

subjected to twice weekly aspiration of follicles =2 mm diameter.

MATERIALS AND METHODS

Animals

Two groups of heifers (n=24); 15 month old with an average live weight of 390±18 kg and 24 month old with an average live weight of 548±11 kg were used in this experiment. During the experiment, all animals were kept in pens and fed hay supplemented with concentrates twice daily, as in normal farm practice. Fresh water was provided *ad libitum*.

Prior to the experiment, all heifers had their oestrous cycles synchronized by two synthethic prostaglandin (Chloprosterol™ and Estrumate™; Pitman-Moore, Australia) injections (2.0 ml, i.m.) given four weeks and two days prior to the first aspiration.

Experimental Design

In the experiment, heifers within each age group were allocated at random to three different treatment groups:

1. Control group (C, n=7, 3x15 month, 4x24 month) were treated with a placebo and received 2,000 IU PMSG at week 4.
2. PMSG group (P, n=7, 3x15 month, 4x24 month) were administered 750 IU PMSG (Pregnecol™ Horizon Australia, i.m.) weekly for the first 4 week of the experimental period and 2,000 IU PMSG for weeks 4.5 to 6, and
3. Inhibin group (I, n=10, 5x15 month, 5x24 month) were actively immunized with 250 μg recombinant ovine inhibin-a3 (r-oIHH-a3, Biotech Australia, s.c.) 4 weeks prior to and 250 μg at the beginning of the experimental period.

Antibody Binding Assays

Antibody inhibin concentrations were measured in duplicate samples of serum from control and immunized heifers by incubating ^{125}I-radiolabelled pure bovine inhibin synthetic peptide with serum at a 1:200 and 1:50 final dilution. Bound and free tracer were separated using 12 % (w/v) polyethylene glycol. Radioactivity of antibody-bound tracer contained in the

pellet was measured using a gamma counter.

Ultrasonographic Examinations

Ovarian follicular dynamics were examined twice weekly for two weeks prior to the six weeks treatment period using an ultrasound equipped with a 6.5 MHz human endovaginal probe (PV6-601V Toshiba Capasee, Japan), extended to a length of 50 cm, with a special grip. This probe was equipped with a 17 gauge needle. At the opposite end, the needle was connected to a 50 ml test tube then to the suction pump unit (VMAR 4000, Cook Vet. Australia) with adjustable negative pressure control. An aspiration pressure of 50 mmHg was used during this experiment.

Oocyte Isolation and Maturation

In the laboratory, all viable COC's (A=>4 layers granulosa, B=3-4 layers & C=1-2 layers, not D=denuded or E=expanded) were cultured for 22-24 hours in four well nunclon multidishes with approximately 10 COC's per well in 0.5 ml TCM-199 covered with mineral oil in a humidified atmosphere of 5 % CO_2 in air before insemination with sperm in microdrops.

Sperm preparation and* In Vitro *Fertilization

Frozen semen (sperm concentration is $2x10^7$ per 0.25 ml straw) was obtained from the Victorian Artificial Breeders (Bacchus Marsh, Victoria). Straws were thawed in water a bath at 37-39 °C for 10 seconds, pooled and prepared for sperm capacitation (Ord *et al.*, 1990).

The COC's were removed from TCM-199 after 22 to 24 hours and inseminated with sperm at $2x10^6$ sperm/ml. The COC's plus sperm were incubated at 39 °C in a humidified atmosphere of 5 % CO_2 in air for 18 hours.

In Vitro *Culture*

Eighteen hours after insemination, the COC's were vortexed in TALP-Hepes (0.5 ml for 1 min 30 sec) to remove the cumulus cells, and placed in Synthetic Oviduct Fluid (SOF) medium (Tervit *et al.*, 1977). Up to four oocytes placed in 30 µl droplets SOF covered with 3,0 ml mineral oil and cultured at 39 °C in 5 % CO_2 in air. Cleaved embryos were placed in fresh SOF after three days and again two days later. After seven days morulae and blastocysts judged to be of good quality were cell spread or a subset transfered to recipient animals.

Measurements and Statistical Analysis

In these experiments, for each animal the following measurements were taken follicle numbers and size at each aspiration, oocyte numbers and quality, and antibody response for I heifers.

Follicle number, oocyte recovery rates and viability of oocytes as determined by grading at collection were analyzed by the restricted maximum likelihood (REML) method giving means and least significant differences (LSD).

RESULTS AND DISCUSSION

Inhibin Antibody Titres

All 10 inhibin-immunized heifers showed high titres for inhibin antibody following the booster injection, and then remained high for the duration of the experiment (bleed 7=6 weeks after booster). The average % binding of inhibin following the booster injection in this group was 41.3 %. Titres in the control heifers (data not shown), remained similar to blank values.

Ovarian Response

During the pre-treatment period, there was a mean of 14.4 follicles =2 mm on the ovaries (10.4 x 2-4, 3.0 x 5-9 and 1.0 x 10 mm). The total numbers of follicles =2 mm was similar in all groups (C=17.0, P=16.0, I=17.6, P>0,05). There was no significant difference in small (2-4 mm), medium (5-9 mm) or large follicles (=10 mm) between the treatment groups in comparison with controls. Similarly, total follicles aspirated, oocytes collected and follicle score (total number x diameter, C=45.7, P=46, I=97, P<0.01) were similar in the three groups.

During the treatment period, following the booster injection inhibin-immunized heifers had significantly more medium-sized follicles (5-9 mm) in comparison with PMSG and controls (C=4.2, P=4.1, I=7.1, P<0.001, Table 1). Similarly, the number of large follicles (=10 mm) was significantly greater in immunized than in PMSG and control heifers (C=1.1, P=1.4, I=4.4, P<0.001) which enabled more follicles to be aspirated from heifers in this group (C=7.9, P=7.4, I=12.6, P<0.01). Inhibin-immunized heifers also had a greater follicle score (C=45.7, P=46, I=97, P<0.01) than PMSG and control heifers. However, in comparison with PMSG

and controls, inhibin-immunized heifers had a decreased mean number of small follicles (2-4 mm) (C=11.6, P=10.5, I=6.1, P<0.05) resulting in a similar number of total follicles =2 mm between all groups (C=17.0, P=16.0, I=17.6). There were no differences between control and PMSG treatments for any of the parameters measured.

Table 1. Mean Number of Small (2-4 mm), Medium (5-9 mm) and Large (=10 mm) Follicles in C, P, and I Following Immunization against Inhibin and Stimulation with PMSG.

Follicle's size	Control	PMSG	Inhibin	LSD1	LSD2
= 2-4 mm CP	10.8	10.4	9.9	2.45	2.26
TP	11.6	10.5	6.1	2.45	2.26
LSD1(P=0.05)	1.71	1.71	1.43		
= 5-9 mm CP	2.8	2.4	3.2	1.89	1.75
TP	4.2	4.1	7.1	1.89	1.75
LSD1(P=0.05)	1.79	1.79	1.50		
= 10 mm CP	0.9	1.1	0.9	1.13	1.05
TP	1.1	1.4	4.4	1.13	1.05
LSD1(P=0.05)	0.95	0.95	0.79		

LSD1: Least significant differences for comparing control with PMSG, LSD2: Least significant differences for comparing Inhibin with control, or Inhibin with PMSG. CP= control period, TP= treatment period

Even though the immunization against inhibin enabled more follicles to be aspirated, inhibin immunization significantly decreased the proportion of oocytes recovered per aspirated follicle (C=0.37, P=0.34, I=0.19, P<0.03, Table 2) in comparison with PMSG and controls. Similarly, inhibin immunization significantly decreased (C=0.30, P=0.27, I=0.16, P<0.02, Table 2) the recovery rate of viable oocytes per aspirated follicles although the proportion of viable oocytes from total oocytes collected were similar for all groups (C=0.81, P=0.79, I=0.84).

Active immunization of heifers against inhibin increased the number of medium (5-9 mm) and large (=10 mm) follicles in heifers undergoing a twice weekly oocyte pick-up program. These results support previous observations in dairy and beef heifers (Glencross *et al.*,1992; Moris *et al.*, 1993; Scanlon *et al.*, 1993) and sheep (Cummins *et al.*, 1986) results in an increased ovulatory response and an increase in the number of large ovarian follicles.

The total number of follicles =2 mm in diameter in the ovary were similar for all treatments. This was a consequence of the decrease in the number of small follicles (2-4 mm) in inhibin-immunized heifers. These results suggest that immunization against inhibin stimulated growth of these small follicles thus pushing them into the larger size follicle classes. It seems

that immunization against inhibin has stimulated follicular growth at about the two milimeter size class, but not stimulated follicles of a smaller size, otherwise we may have expected the number of follicles 2-4 mm to have remained the same or to have increased.

Table 2. Proportion of oocytes and viable oocytes per follicle aspirated in C, P, and I following immunization against inhibin and stimulation with PMSG.

Total oocytes/aspirated	Control	PMSG	Inhibin	LSD1	LSD2
Total oocytes CP	0.51	0.40	0.45	0.13	0.12
TP	0.37	0.34	0.19[a)]	0.13	0.12
LSD(P=0.05)	0.129	0.129	0.108		
Viable oocytes CP	0.35	0.25	0.36	0.13	0.12
TP	0.30	0.27	0.16[b)]	0.13	0.12
LSD(P=0.05)	0.135	0.135	0.113		

LSD1: Least significant differences for comparing control with PMSG, LSD2: Least significant differences for comparing Inhibin with control, or Inhibin with PMSG. CP= control period TP= treatment period

Collectively, these results indicate that active immunization against inhibin may not affect ovarian follicle development through increases in FSH concentrations and that there may be a direct effect at the ovarian level. To support this hypothesis, some evidence suggest that inhibin or their subunits may have local effects on gonadal function (Tsafriri *et al.*, 1989; Woodruff *et al.*, 1990).

The percentage of viable oocytes (A, B, and C class) collected from any treatment groups (C, P, and I) was similar in this experiment, thus changes in follicular growth in the inhibin group did not effect oocyte quality. Further support is evidenced by 25 % of viable oocytes reaching morulae and blastocysts stage by day 7 being similar for all treatment groups.

CONCLUSIONS

The present study demonstrates the potential of active immunization against inhibin to increase the number of follicles aspirated in heifers undergoing the oocyte pick-up program through a sustained increased follicular development. The mechanism by which inhibin does this may be at the ovarian level rather than effecting peripheral gonadotrophin concentrations.

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