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Effect on reproductive function in sheep and cattle of inhibin immunoneutralization

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Summary — Inhibin is a glycoprotein hormone secreted, in the female, by developing ovarian follicles. When administered to anoestrus ewes or ovariectomized heifers, when partially- or highly-purified from bovine follicular fluid, it selectively depresses plasma follicle-stimulating hormone (FSH) concentrations (and therefore probably pituitary FSH secretion) in a dose-dependent manner. As FSH is responsible for promoting follicular growth, the neutralization of endogenous inhibin action by antibodies should therefore maintain or increase FSH secretion and stimulate follicular growth. In experiments to test this, it was observed *via* laparoscopy, that active immunization against a synthetic peptide fragment of bovine inhibin during the late anoestrous period of ewes resulted in a 90% increase in ovulation rate in the subsequent breeding season and a 35% increase in lambing rate following breeding. Using ultrasonography, it was observed that similar immunization of heifers beginning in the prepubertal period (4 months of age) resulted in a 35% increase in the incidence of multiple ovulations (mostly twin). The data demonstrate the potential of inhibin immunoneutralization in obtaining a controlled increase in ovulation rate in sheep and cattle and an increased lambing rate. The effect on calving rate has yet to be determined.

inhibin / FSH / ovulation rate / litter size / immunization

Résumé — Effet de l'immunisation contre l'inhibine sur la fonction de reproduction des ovins et des bovins. *L'inhibine est une hormone glycoprotéique sécrétée, chez la femelle, par les follicules ovariens en croissance. Lorsque l'hormone, partiellement ou hautement purifiée à partir de fluide folliculaire bovin, est administrée à des brebis en anœstrus ou à des génisses ovariectomisées, elle diminue les concentrations plasmatiques de FSH (et donc probablement la sécrétion de FSH hypohysaire) dans des proportions qui dépendent de la dose administrée. Puisque la FSH facilite la croissance folliculaire, la neutralisation de l'inhibine endogène par des anticorps doit donc avoir pour résultat le maintien ou l'accroissement de la sécrétion de FSH et la stimulation de la croissance folliculaire. Dans les expériences mises en place pour tester cette hypothèse, l'endoscopie a démontré que l'immunisation active contre un peptide synthétique, fragment de l'inhibine bovine, pendant la fin de la période d'anœstrus chez la brebis, a eu pour résultat une augmentation de 90% du taux d'ovulation pendant la saison sexuelle suivante et de 35% de la prolificité après la mise en reproduction. De la même façon, on provoque l'immunisation de génisses pendant la période prépubère (à l'âge de 4 mois) un accroissement de 35% (visible grâce à l'échographie) du taux d'ovulations multiples (doubles pour la plupart). Les résultats démontrent les potentialités de l'utilisation de l'immunoneutralisation de l'inhibine dans le but d'obtenir une augmentation contrôlée du taux d'ovulation chez les ovins et bovins et un accroissement du taux d'agnelage. L'effet sur le taux de vêlage reste à déterminer.*

inhibine / FSH / taux d'ovulation / taille de portée / immunisation

Inhibin is a glycoprotein hormone synthesised, in the female, by the granulosa cells of ovarian follicles and secreted during the antral stage of follicular development. Highly-purified inhibin from bovine follicular fluid (Fukuda *et al,* 1986; Robertson *et al,* 1986; Knight *et al,* 1990), when administered to ovariectomized ewes (Findlay *et al,* 1987) or heifers (Beard *et al,* 1990) selectively depresses pituitary follicle-stimulating hormone (FSH) secretion and plasma FSH concentrations. FSH is an important gonadotrophic hormone responsible not only for promoting the growth of follicles but, it is believed, for the selection of follicles for ovulation.

The neutralization of endogenous inhibin action by antibodies should therefore maintain or increase FSH secretion, stimulate increased follicular development and possibly increase the number of follicles ovulating.

The bovine inhibin molecule consists of 2 dissimilar polypeptide chains, the α and β subunits, joined by disulphide bridges. The part of the molecule conferring hormone specificity resides in the larger α subunit.

Following DNA analysis (Forage *et al,* 1986), peptide sequences have been synthesised. Preliminary studies showed that a synthetic peptide comprising the N-terminal, 1-29 amino acid sequence of the α subunit of bovine inhibin, when conjugated to carrier proteins to provide immunogenicity (purified tuberculin protein derivative or ovalbumin) produced antibodies in sheep and cattle, capable of binding labelled native bovine inhibin. We have therefore used this synthetic 1-29 inhibin fragment to investigate the effect of inhibin immunoneutralization on plasma FSH concentrations and reproductive function in sheep and cattle.

Both Romney (n = 20) and Mule (Blue-faced Leicester x Swaledale) ewes (n = 29), when actively immunized against this synthetic peptide fragment of bovine inhibin over an 8–10 week period starting within seasonal anoestrous and continuing into the breeding season (Wrathall *et al,* 1990, 1992) showed elevated inhibin antibody titre in plasma (of the order of 15–20% at 1:1 000 final dilution), as measured by the binding of iodine-labelled native bovine inhibin. Plasma FSH concentrations, measured by a specific ovine FSH radioimmunoassay, were slightly higher in these treated ewes when antibody titre was elevated. Examination of ovaries for corpora lutea by laparoscopy after synchronization of cycles (using progesterone sponges) when in the breeding season, showed an almost doubling of ovulation rate from 1.05 to 2.15 ovulations/Romney ewe and 2.07 to 3.9 ovulations/Mule ewe. Furthermore, when the Mule ewes were naturally served at the next oestrus, all conceived and lambing rate was increased from 1.97 to 2.66 lambs/ewe. Oestrous cycle and gestation lengths were unaffected.

Friesian heifers (n = 6) similarly immunized from 4–18 months of age (fig 1) showed increased plasma antibody titre (15–30% at 1:2 000 final dilution) before puberty and throughout subsequent cyclic ovarian activity (fig 1). Plasma FSH concentrations fell gradually during the prepubertal period. They were slightly more elevated in treated heifers around the time of puberty and over the period of cyclic activity (fig 1).

The onset of puberty, defined as d 1 of the first cycle that had a normal luteal-phase plasma progesterone concentration, occurred at a similar age and body weight in control and treated heifers. The incidence of multiple ovulations (mostly twins), observed as the number of corpora lutea by transrectal ovarian ultrasonography was 35% higher ($P < 0.001$) in treated heifers (table I). There was a 40% increase in ovulation rate (1 to 1.4 ovulations/cycle).

This preliminary work has shown that active immunization against inhibin can increase ovulation rates and lambing rates in sheep. It can also increase ovulation rates

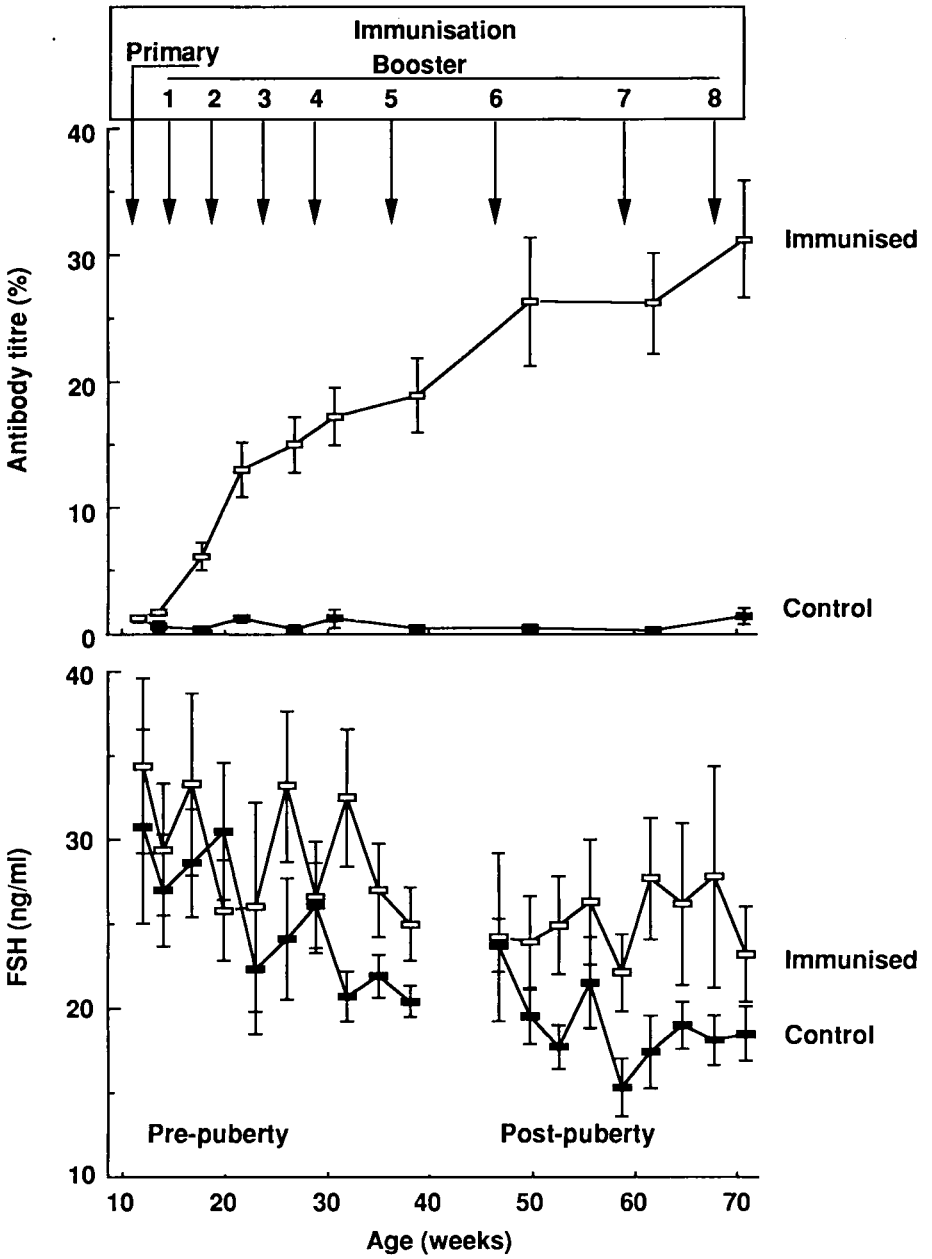

Fig 1. Time of immunization, mean antibody titre (± SEM), measured as % binding of iodine-labelled native bovine inhibin in plasma (1:2 000 final dilution) and mean plasma FSH concentrations (± SEM), measured using a FSH assay with bovine FSH standard (USDA, B1) labelled-trace (USDA, BP3) and ovine antisera, in control (n = 6) and treated (n = 6) heifers at 3 weekly intervals froms 4–18 months of age.

Table I. Number of ovulations, measured as numbers of corpora lutea by transrectal ovarian ultrasonographic observation, in 6 immunized heifers from cycle 5–14 post-puberty. A single ovulation only was observed in all 5 cycles over the same period in 6 control heifers.

Immunized heifer No	*Cycle No*									
	5	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*
2	1	1	1	2	1	3	1	2	3	3
3	–	1	1	1	1	2	1	1	3	1
6	1	2	1	1	2	1	1	1	2	1
7	2	1	2	1	2	1	2	1	4	2
8	–	1	1	1	1	1	2	1	2	–
11	1	1	2	1	0	1	1	1	1	–

in cattle. Its effect on conception, pregnancy and calving has yet to be determined in cattle. Its impact on sheep production will probably not be as great as on cattle production, since other methods of increasing ovulation numbers such as selective breeding, using the Booroola gene, and immunization against steroids, notably androstenedione, are practised. It does at present, however, appear to be the only reliable method of inducing multiple ovulations, particularly twins, in cattle.

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