MULTI-STEROID IMMUNIZATION - A PRACTICAL TREATMENT TO INCREASE FECUNDITY IN MERINO EWES

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SUMMARY

Immunization of Merino ewes with three steroid-protein conjugates given simultaneously was investigated to develop an improved vaccine mixture suitable for primary and booster treatments to increase fecundity. Using two types of multi-steroid mixtures, the required immune responses were achieved when the oestrone immunogen component was reduced. In the first year in two trials, ewes immunized with these modified mixtures had a greater lambing percentage than untreated ewes. Immunization against the single steroid dehydroepiandrosterone also increased lambing percentage. Gains were not always achieved with androstenedione immunization and in two trials multi-steroid vaccination was more effective than androstenedione. Lambing increases of up to *35%* were obtained with these improved mixtures of steroid immunogens.

INTRODUCTION

In Merino ewes, the use of multiple steroid immunization to increase fecundity has proved superior to androstenedione immunization (Fecundin^{R}) probably due to better survival of ova and embryos (Wilson *et al.* 1986). In preliminary trials with multiple steroid immunogens, the individual components of the mixture were varied in the primary and booster injections. This was necessary to achieve the desired steroid antibody levels as immunization with mixed immunogens results in interactions which affect the antibody responses (Taussig 1977; Cox *et al.* 1988). For optimal fecundity, immunogens for each booster injection were selected *to* give moderate androstenedione (A), testosterone (T) and oestrone (E) antibody titres (generally from 1:200 to 1:6000), with the E titres being lower than the androgen titres to minimise anoestrus (Cox *et al.* 1988). Although this approach was successful, a more practical system is needed with an identical mixture for primary and subsequent immunizations.

The current studies were undertaken in order to develop suitable vaccines giving defined and consistent antibody responses following each booster and in particular to control the levels of E antibodies. These vaccines were then evaluated for their effectiveness in improving the reproductive performance of Merino ewes. Responses following single-steroid immunization against androstenedione or dehydroepiandrosterone (D) were also determined.

MATERIALS AND METHODS

Steroid-protein immunogens of A, T, E or D conjugated to human or bovine serum albumin (HSA or BSA), were dissolved or suspended in 5% DEAE-dextran/0.45% saline. Sheep *were* immunized with the vaccine mixtures by subcutaneous injection at two sites, with 1.2 mg (unless otherwise stated) of each immunogen in 2 ml adjuvant. Groups of 6 to 10 sheep were used for testing immunogen responses, with booster immunizations of the same mixtures being given *3* and 12 weeks after the primary. Blood was collected 1 week after each boost. Antibody titres were determined as the reciprocal dilution of serum showing 50% of maximal binding of tritium labelled steroids, and expressed for a group of sheep as geometric mean titre (back transformation of mean log titre).

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Table 1 Immunogens and mixtures used for assessing variations in antibody titre and for field trials

Mixture type			UH			UB		
Immunogens	A.HSA	D.HSA	A.HSA	T.HSA	E.HSA	A.HSA	T.HSA	E.BSA
Weight ratio of immunogen								
1. Mixture test	–	–	1	1	1	1	1	1
			1	1	0.05	1	1	0.25
			1	1	0.01	1	1	0.1
			1	1	0.002	1	1	0.04
2. Trial A	1	–	1	1	0.02	1	1	0.5
3. Trial B	1	1	–	–	–	1	1	0.25

Two types of steroid-protein mixtures were chosen for examining variations in antibody titres (Table 1); these were mixtures of three immunogens with A and T conjugated to HSA, and E conjugated to either HSA (UH type mixture) or BSA (UB type mixture). The same A and T conjugate preparations were used for both these mixtures. The proportion of E immunogen present in each type of mixture was varied and the resulting antibody titres measured.

Based on these results, selected mixtures (Table 1) were used in two trials with fine-wool mature Merino ewes to assess the effects on oestrus, ovulation and lambing rates. These trials were held for two years at Armidale, (Trial A) and for one year at Badgery's Creek (Trial B). Ewes were randomly allocated according to live weight to four groups (n=50–69); an untreated control group (C), an A.HSA immunized group (A), a group immunized against a UB mixture (UB) and a fourth group in Trial A immunized against a UH mixture (UH) and in Trial B against D.HSA (D). In Trial B, the UB mixture was used at one third the usual dose. The ewes were immunized 7 and 4 weeks before being joined with entire rams (2%) for 5 weeks. In the second year for Trial A, re-immunization was 4 weeks before joining. Ovulations were determined by laparoscopy of ewes following weekly oestrous observations. The number of lambs was determined at birth *or* from ultrasound scanning of ewes at 50-80 days gestation.

RESULTS

Immunogen mixtures

In mixtures when equal proportions of the three immunogens were present (i.e. when E immunogen ratio was 1), some suppression of individual antibody responses occurred but E antibody titres remained higher than A or T titres particularly in the UH mixture *(Fig.* 1).

A lowering of E antibody titres was obtained when the ratio of E immunogen *in* the mixtures was reduced below 1. This had different effects on A and antibody titres in the two types of mixtures. In UH mixtures, A and T antibody titre levels were inversely related to E antibody levels, while in UB mixtures all titres decreased but E titres more rapidly than T or A. With further boosting, the pattern and level of the various antibody responses were similar.

Vaccines were considered to be suitable if moderate to low immune responses, with E titres preferably lower than A or T, were obtained after each booster. This was best achieved with the UH mixture with ratios of A,T and E immunogens of 1:1:0.05 and with the UB mixture with ratios of 1:1:0.1. The mixtures used in the field trials had slightly different ratios due to the different steroid content of the immunogens but gave similar titres.

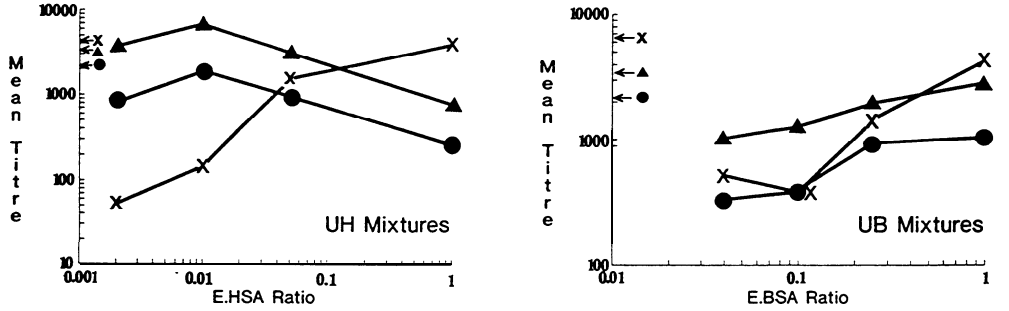

Fig. 1. Antibody titres against A (●), T (▲) and E (X) following two immunizations with UH or UB mixtures having different ratios of E immunogens. Arrows indicate titres of single steroid immunogens

Field evaluation

Table 2 Field trials of vaccines to assess immunity and fecundity responses

Trial	Group immune to	No. ewes	Antibody titres to A	T	E	D	% Oestrus	O/EJ+	No. lambs/ewe 0	1	2	3	L/EJ+
Trial A													
1st year	C	63	-	-	-	-	98	1.40	10	36	17	0	1.11
	A	65	600	-	-	-	95	1.82***	10	24	26	5	1.40*
	UH	64	110	360	110	-	97	1.79***	6	28	29	1	1.39*
	UB	68	120	330	120	-	100	1.72***	4	33	30	1	1.41**
2nd year	C	65	-	-	-	-	97	1.78	8	24	32	1	1.40
	A	68	880	-	-	-	96	1.90	15	24	28	1	1.22
	UH	68	240	550	240	-	96	1.96	10	18	38	2	1.47
	UB	69	470	950	240	-	99	1.93	5	24	39	1	1.52
Trial B													
1st year	C	50	-	-	-	-	100	1.34	6	34	10	0	1.08
	A	64	3800	-	-	-	97	1.72***	15	31	18	0	1.05
	D	65	-	-	-	9010	95	1.97***	11	22	31	1	1.34*
	UB	65	2240	7300	640	-	98	2.09***	9	22	31	3	1.43**

+ O/EJ=ovulations per ewe joined; L/EJ=lambs per ewe joined, and in Trial B foetuses per ewe joined.
* P<0.05, **P<0.01, ***P<0.001, compared to C; Chisquare test (Brown 1988)

In Trial A, ewes immunized with the UH and UB mixtures gave the desired low antibody responses (Table 2). In the second year of treatment, similar titre levels were again obtained. At joining the mean live weight of the ewes was 43kg in the first year, and 48 kg in the second year due to exceptionally good seasonal conditions. All ewes showed normal oestrous behaviour in both years. The ovulation rates of UH, UB and A immune ewes were higher than for control ewes (Table 2); this difference was significant in the first year. In the second year the seasonal conditions resulted in a higher than usual ovulation rate of 1.78 in the control ewes and a smaller difference between these and immune ewes. The number of lambs born per 100 ewes in the first -year was significantly greater in the A, UH and UB immune groups than in the control group. In the second year the untreated ewes had a high lambing rate, and compared with this group the lambing responses of the immune groups were not significantly different. However, the UB immune ewes had significantly more

lambs and a lower incidence of dry ewes than the A immune ewes ($P < 0.05$), the lambing percentage of the latter being less than the controls.

In Trial B, with the smaller dose of UB mixture, E titres were lower than A or T but the overall immune responses were high. The season was unusually good but very wet during joining; mean live weight at joining was 43 kg. Oestrous behaviour was normal and all immune groups had significantly higher ovulation rates. Again the lambing rate of the A immune group was lower than expected in contrast to the other immune groups ($P<0.05$). Both D and UB groups had significantly better lambing response than the control group with UB having the highest gain of 35% in lambs born.

DISCUSSION

Although immunization against mixtures of steroids gives a range of immune responses, the results show that suitable multi-steroid vaccines can be designed to obtain antibody titres within a defined range using the same formulation for each booster treatment. By reducing the proportion of E imxnunogen in the mixtures, a low E antibody response is obtained, thereby avoiding an increase in the occurence of anoestrus. In Merino ewes, increases in ovulation rate resulted with both vaccine mixtures, while the UB mixture gave better lambing responses. Improvements in lambing performance were achieved with a smaller dose of the vaccine but further adjustment of the dosage may be required to obtain the preferred lower immune responses.

Immunization of Merinos against Fecundin has *given* variable lambing results particularly when seasonal conditions were favourable (Cox *et al.* 1988). This is seen in two of the current tests, and in contrast to Fecundin, multi-steroid immunization resulted in 25 to 38% more lambs and fewer dry ewes. The conditions in the second year of Trial A would not usually beencountered as untreated ewes had an exceptionally high ovulation rate of 1.78 and a lambing rate of 1.40. In these circumstances the immunization of ewes to improve lambing performance was expected to give an indication of the effects under conditions of high nutritional intake and multi-steroid immunization proved more effective than Fecundin. Immunization against dehydroepiandrosterone which has given ovulation rate increases in cattle (Hanley *et al.* 1988) also resulted in a significant increase in lambing percentage with similar but lower responses to that obtained with multi-steroids.

By defining the interactions of the various steroid immunogens, suitable multi-steroid vaccines have been developed which provide a practical immunization treatment especially for Merino ewes resulting in gains in lambs born.

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