EFFECT OF REPEATED ANNUAL MULTI-STEROID IMMUNISATION ON THE REPRODUCTIVE PERFORMANCE OF FINE-WOOL MERINO SHEEP

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SUMMARY

In fine-wool Merino ewes, immunisation against a mixture of steroids was compared with androstenedione immunisation alone and with no treatment to evaluate its long term effectiveness in improving reproductive performance by increasing fecundity. Using 2 prototype multi-steroid vaccines in field trials conducted for 4 consecutive years, gains in lambing percentage were consistently obtained, with 1 of the 2 vaccines proving slightly superior. Little variation occurred in the immune responses obtained with these mixtures and there was no reduction in the percentage of ewes joined. Gains in fecundity were not always achieved with androstenedione immunisation, although it can be equally effective as multi-steroid immunisation in some seasons.
Keywords: steroids, fecundity, immunisation, Merino ewes, fine-wool.

INTRODUCTION

In Merino ewes better gains in lambing percentage have been achieved by simultaneous immunisation against a combination of steroids than by androstenedione immunisation, apparently due to improved ova and embryo survival (Wilson *et al.* 1986). This has led to the development of prototype multi-steroid vaccines suitable for practical application to improve reproductive performance (Cox *et al.* 1988; Wilson *et al.* 1990). For optimal fecundity, the multi-steroid vaccines immunise against androstenedione (A), testosterone (T) and oestrone (E), and the steroid antibody responses must be moderate (1: 100 to 1:3 000), with the oestrone titre preferably lower than the androgen titres to minimise the incidence of anoestrus (Wilson *et al.* 1991).

Field evaluation of multiple steroid vaccines has shown that gains in lambing percentage occur when ewes are immunised for 1 or 2 consecutive seasons (Wilson *et al.* 1990). However, it has not been established whether repeated annual use of such treatments during the reproductive life of the ewe will continue to prove effective in increasing fecundity. Further, changes in immune responses associated with various steroids may occur with repeated annual imrnunisation and may affect optimal reproductive performance.

The current studies were undertaken to evaluate the effect of annual immunisation against steroids over 4 years on the reproductive performance of fine-wool Merino ewes. Untreated ewes were compared with ewes immunised against androstenedione (Fecundin) alone or against 2 differing multi-steroid vaccine mixtures. The suitability of the vaccines to give defined and consistent antibody responses was also evaluated.

MATERIALS AND METHODS

For imrnunisation, steroid-protein immunogens of A, T, E conjugated to human or bovine serum albumin (HSA or BSA) were dissolved or suspended in 5% DEAE-dextran in 0.45% saline. The sheep were immunised by subcutaneous injection with 1.2mg of each immunogen, unless stated otherwise, in a total of 2ml DEAE-dextran adjuvant. Two types of multi-steroid vaccines were selected for their differences in relative composition, with 1 vaccine having identical protein carriers, and the other vaccine 2 differing carriers. Each vaccine comprised a mixture of 3 immunogens, with A and T conjugated to HSA, and E conjugated to either HSA (UH mixture) or BSA (UBmixture). In the UH mixture the A, T and E immunogens were in the weight ratio of 1: 1:0.02, and in the UB mixture, 1: 1:0.5. These mixtures were used for sheep in group UH and UB respectively. Blood from 10 sheep per treatment group was collected 1 week after each boost. Antibody titres were determined as the reciprocal dilution of plasma showing 50% of maximal binding of tritium labelled steroids, and expressed for a group of sheep as the geometric mean titre.

Fine-wool Merino ewes, initially 3 years old, were used in field trials conducted at Armidale NSW over 4 consecutive years from 1988 to 1991. The ewes were randomly allocated according to liveweight to 4 groups (initially $n = 70$); an untreated control group (C), an A:HSA immunised group (A), and 2 groups each immunised against a differing multi-steroid mixture (UH and UB respectively). The ewes were immunised at 7 and 4 weeks before joining with entire Merino rams (2%) for 5 weeks. In the second and subsequent years, a single booster immunisation was given 4 weeks before joining.

Harnesses and crayons were fitted to the entire rams to detect oestrus and mating; following weekly oestrous observations, the ovulation rate was determined by laparoscopy of the marked ewes. The number of foetuses present was determined from 2 ultrasound scans of ewes at 50–75 days gestation. The 4 groups were lambed separately, and the number of lambs born was recorded daily. For statistical analysis, Student's t-test was used for antibody titres, and χ^2 analyses (Brown 1988) for reproductive parameters.

RESULTS

During the 4-year period, the seasonal conditions prior to and during joining (approximately February to May) were above average in 1988 and 1989, very wet in 1990, and dry in 1991. The mean liveweights of the ewes at the time of joining were 43, 48, 43 and 45 kg for years 1 to 4 respectively (43 kg is typical average liveweight for this strain of sheep in this location). In the first 2 years, there were very few ewe deaths; however, considerable losses occurred during the 1990 lambing period, with unseasonal weather contributing to pregnancy toxaemia or hypothermia off shears (39 deaths in total) and poor nutrition (6 deaths). The losses were similar for all groups.

Imrnunisation against single or multiple steroids gave antibody responses in the range of 1: 100 to 1: 1200, and the E titres remained lower than the combined androgen titres following each booster treatment (Table 1). While both the UH and UB mixtures produced similar antibody titre responses each year, the E titres of the UB ewes had increased by the fourth year of the trial. In each year, all groups showed a negligible disturbance of oestrus. The ovulation rates of A, UH and UB ewes were significantly higher than for the control ewes in the first and fourth years. The abundance of feed in the second year elevated liveweights, resulting in an unusually high ovulation rate for the control ewes (1.78), with no significant difference between these and immune groups. In the third year, immunisation treatments increased ovulation rates but this was significant in only UB immune ewes.

Table 1. Antibody titres and reproductive performance of control and immunised Merino ewes over 4 consecutive years

Ovulations per ewe joined (O/EJ), ewes lambing/ewes mated or in year 4 ewes pregnant/ewe joined (fertility), lambs born per ewe lambing (LB/EL) in year 4 foetuses/ewe pregnant (F/EP), lambs born per ewe mated (LB/EJ), in year 4 foetuses per ewe mated (F/EJ)

Group	No. of ewes	Antibody titres to A	T	E	Oestrus (%)	O/EJ	Fertility	LB/EL	F/EP	LB/EJ	F/EJ
					Year 1[A]						
Control	63	—	—	—	098	1.40	84	1.32		1.11	
A	65	600	—	—	095	1.82***	85	1.65**		1.40*	
UH	64	110	360	110	097	1.79***	91	1.53*		1.39*	
UB	68	120	330	120	100	1.72***	94	1.50		1.41**	
					Year 2						
Control	65	—	—	—	097	1.78	88	1.60		1.40	
A	68	880	—	—	096	1.91	78	1.57		1.22	
UH	68	240	550	240	096	1.96	85	1.72		1.47	
UB	69	470	950	240	099	1.93	93	1.64		1.52	
					Year 3						
Control	64	—	—	—	100	1.26	94	1.15		1.08	
A	68	1190	—	—	099	1.40	85	1.31*		1.12	
UH	66	190	360	290	098	1.42	92	1.31*		1.21	
UB	69	390	590	570	099	1.58***	91	1.44***		1.32*	
					Year 4						
Control	53	—	—	—	100	1.42	87		1.35		1.17
A	59	880	—	—	097	1.78***	85		1.62**		1.37
UH	51	220	440	300	098	1.84***	86		1.61**		1.39
UB	55	450	790	920	100	1.87***	93		1.65**		1.53**

[A]In Year 1, a few ewes in each group were found to be pregnant prematurely, and have not been included.
$*P < 0.05$, $**P < 0.01$, $***P < 0.001$, compared to control.

The fertility of the treated ewes did not differ significantly from that of control ewes in each of the 4 years, although there was a greater variability following A immunisation, with more dry ewes present in year 2 and 3 (Table 1). In the 4 year period, the occurrence of dry ewes was generally lowest in the UB immune ewes, and this was a significant improvement on the performance of the A immune ewes ($P<0.01$; Fig. 1). The effects of ovulation rate differences and fertility were reflected in the number of lambs born per 100 ewes joined, with the A, UH and UB ewes having higher lambing percentages than the control ewes in the first year ($P<0.05$). In the second year, differences between the lambing responses of the control and multi-steroid immune ewes were not significant as the untreated ewes had such a high lambing rate. However, the UB immune ewes had significantly more lambs than group A ($P<0.05$) due to the latter having a higher incidence of dry ewes and fewer lambs than untreated ewes. In years 3 and 4, while more lambs or foetuses were present in the immune groups, only the UB group showed a significant increase in the number of progeny per ewe joined.

In most instances, the A, UH and UB immune ewes had a higher prolificacy (number of lambs per ewe lambing) than the untreated ewes, with the exception of year 2 when the controls also had an unusually high level of prolificacy. The increase in prolificacy in these groups was associated with a higher proportion of twin births than in the control ewes (Fig. 1). Yearly differences in litter size were similar for all groups, and over the 4 years, the UB group tended to have more twins than the other groups. There were very few triplets born to immunised ewes, particularly in the groups UH and UB.

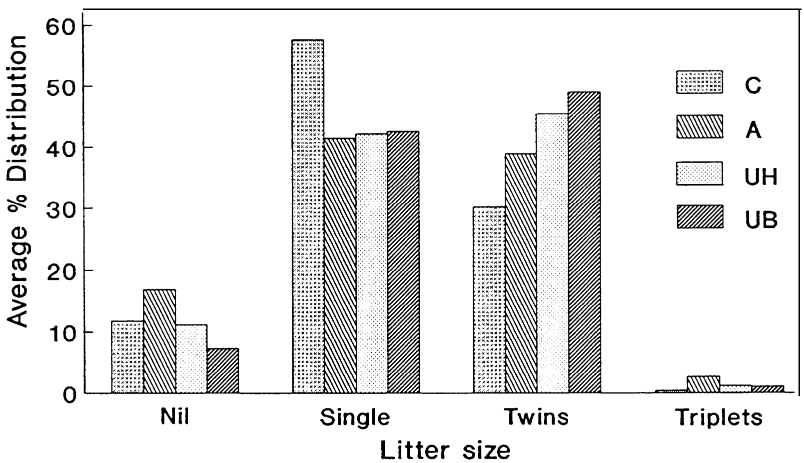

Fig. 1. Average distribution over 4 years of the percentage of ewes with litter sizes of nil, single, twins and triplets for group C, A, UH and UB.

DISCUSSION

In fine-wool Merino ewes, the use of multi-steroid immunisation will result in continuing gains in reproductive performance when used repeatedly in the same ewes for 4 consecutive years. The average increase in lambing percentage over this period was 8, 18, and 25% for sheep immunised against Fecundin, UH and UB multi-steroid vaccines respectively. Of the 2 prototype vaccine mixtures evaluated, the UB mixture was slightly superior as evidenced by the number of lambs born per ewe, as well as by a marginally higher level of fertility in most years. Despite differences in immunogen composition and reproductive performance, the antibody responses of the 2 vaccines were similar. Repeated booster immunisation resulted in little variation in antibody titre levels, and the levels were maintained in the desired range necessary for optimal fecundity. The relatively low proportion of E immunogen in the mixtures proved effective in retaining normal oestrus (Wilson *et al.* 1990). In the latter 2 years, steroid antibody levels in UB ewes, immunised against steroids conjugated to 2 carrier proteins, tended to be higher than in the UH group, and this may possibly contribute to the slightly better reproductive performance of this group. Trials of other multi-steroid mixtures using 2 carrier proteins produced antibody titre levels similar to UB and also resulted in favourable reproductive performance (Croker *et al.* 1991).

These trials confirm that while Fecundin immunisation is applicable to Merino ewes, it may at times give poor results and an increase in dry ewes in this breed (Cox *et al.* 1988; Wilson *et al.* 1990). These disadvantages can be overcome by multi-steroid immunisation using the preferred UB-type mixture. In this and other related trials, the extra multiple births in the immune groups did not penalise the ewes in their annual wool production compared to untreated ewes. The use of this innovative technology, coupled with good management practices to ensure optimal lamb survival, means that producers can now maintain production from fewer breeding ewes. Additional benefits can also be derived by using multi-steroid immunisation to enhance the introduction of new genetic traits to the Merino industry.

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REFERENCES

BROWN, G. H. (1988). *Aust. J. Agric. Res.* 39: 899–905.

COX, R. I., WILSON, P. A., WONG, M. S. F. and PAULL, D. R. (1988). Proc. 11th Intern. Congr. Animal Reprod. and A.I., Dublin. 4: 489.

CROKER, K. P., WILSON, P. A., JOHNS, M. A. and COX, R. I. (199 1). *Anim. Reprod. Sci.* 25: 199-208.

WILSON, P. A., COX, R. I., WONG, M. S. F. and PAULL, D. R. (1986). *Proc. Aust. Soc. Reprod. Biol.* 18: 41.

WILSON, P. A., COX, R. I., WONG, M. S. F. and PAULL, D. R. (1990). *Proc. Aust. Soc. Anim. Prod.* **18: 432-5.**

WILSON, P. A., COX, R. I., WONG, M. S. F. and PAULL, D. R. (1991). *Proc. Aust. Soc. Reprod. Biol.* 23: 55.