APPLICATION OF IMMUNIZATION TECHNIQUES TO IMPROVE LIVESTOCK PRODUCTIVITY

INTRODUCTION

T.W. SCOTT*

During the past two decades we have witnessed an explosion of knowledge in the life sciences and currently scientists are poised to produce a number of new technologies that could have profound effects on the efficiency of livestock production. Our understanding of the many hormones and growth factors that influence critical production areas e.g. reproduction, body and wool growth, has increased significantly. Although much more fundamental research is required to fully appreciate the mechanisms by which these substances influence productive traits, sufficient knowledge exists to allow us to develop technologies that enable the endocrine system to be manipulated with the overall aim of improving productivity. Autoimmunity to hormones exerting a suppressive effect on the expression of genetic potential is such a technology.

The principal themes to be developed in three of the following papers relate to the use of immunophysiological procedures to enhance the number of lambs born to ewes joined. The basic concept involved the development of a vaccine, composed of a steroid-protein immunogen, which provokes anti-steroid antibody and effectively deregulates the hormonal control of ovarian function. This results in an increase in ovulation rate and overall fecundity. The papers describe the development of this process into a reliable practical procedure for increasing lambing percentages.

We believe that this example will be the first of a number of new applications that will be developed in the future. The last paper categorizes immunophysiology (hormone-immunity) research as a developing multidisciplinary facet of biology that will enable us to produce a new range of fascinating biologically active molecules capable of influencing animal productivity. There will be scope to improve body and wool growth, regulate appetite and influence body composition of livestock as a result of applying the principles of immunophysiology to develop novel vaccines.

INCREASED LAMBING PERCENTAGES IN SHEEP USING A CONTROLLED ACTIVE IMMUNIZATION AGAINST STEROIDS

R.I. COX*

The experimental observations in the early 1970's, that increased ovulation rates in sheep could be produced by immunization against some oestrogens or androgens, opened up a completely new area for improving reproductive efficiency (Cox et al. 1976; Scaramuzzi 1976; Scaramuzzi et al. 1977). Immunization techniques offered the potential of an immediate gain in ovulation rate and hence lambing percentages, the flexibility of a decision each season, and the possibility of a simple treatment. This potential required a number of years of developmental research before it was translated into reality and the scope and constraints defined for an immunophysiological control of reproduction. Major difficulties that were overcome included the development of a controlled active immunization process, overcoming over-responses and associated anoestrus and simplification of the immunization protocol. The main objective was to improve the proportion of twins born to

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DEVELOPMENT OF A CONTROLLED ACTIVE IMMUNIZATION SYSTEM

Active immunization was considered likely to be more practical than a passive system if a reproducible and controlled response could be achieved. The immunogen and adjuvant had to be matched to produce a suitable response in antibody level and ovulation rate. Steroid specific antibody levels were used as an index and were found to correlate with ovulation rates. Ovulation rate increases are not necessarily translated into embryos or lambs; nevertheless some increase in lambing percentage was usually achieved with the system developed.

The immunogens. As the steroids are not immunogenic they have to be covalently linked chemically to a suitable immunogenic carrier. The linkage of the steroid to the protein does not appear to be critical (Hoskinson et al. 1981) and various protein carriers are satisfactory. The antibodies produced as a result of treatment with such steroid-protein conjugates combine with a high degree of specificity with the steroid, and this neutralizes or reduces the biological action of the steroid.

Initial studies had shown that increases in ovulation rate could occur following treatment of ewes with protein conjugates of oestrone (Cox et al. 1976), androstenedione (Scaramuzzi et al. 1977), or testosterone (Scaramuzzi 1979; Cox et al. 1982). Similar end results have been obtained with all of these steroids although the mechanisms, yet to be fully elucidated, are likely to be different in detail.

In order to limit the size of tests undertaken, androstenedione-7a-carboxeyethylthioether:human serum albumin (androstenedione-7-HSA) was selected for the greater proportion of tests conducted after 1981.

The adjuvant and the immune response. The immunogens require an adjuvant to stimulate an adequate response. A number of adjuvants producing a wide range of antibody titres was tested. Once it was established what antibody titre was required for a good ovulatory response it was possible to select an adjuvant for more detailed study. A good response was necessary in both an initial year of treatment and associated with the appropriate immune "memory" in subsequent years. Such an adjuvant was found to be DEAE-dextran, a polycationic compound described as useful in viral work by Wittmann (1970) and for steroid-protein conjugates (Cox and Wilson 1976). It has the advantages of being water soluble, and in aqueous solution with the immunogen is readily sterile filtered, resulting in a stable and easily administered treatment which produces minimal tissue reaction.

With this adjuvant the antibody response and titre decline curve (Fig. 1) vary according to the time interval between the two treatment injections. This has proved very useful in selecting the timing of treatment to produce different responses.

Other adjuvants of the water/oil emulsion type, such as Freunds adjuvant, give quite different response curves, with a higher and more sustained antibody titre.

With DEAE-dextran as adjuvant and 3-4 weeks spacing between the treatments, antibody titres were maximal about 1 week following the second treatment and then declined moderately rapidly (Fig. 1). Such a spacing (and hence response) has been found to have several advantages; it produces the
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Fig. 1 The antibody titre responses when there were intervals from 7 to 28 days between the first and second treatment using DEAE-dextran adjuvant. Data for 10 and 14 days and 21 and 28 days have been combined. The test immunogen was oestrone-6-carboxymethyl-oximino:HSA. The antibody titre was measured using H-oestrone (Cox et al. 1982).

highest titres obtainable with a particular immunogen in DEAE-dextran and has resulted in maximal gain in ovulation rate without persistent anoestrus. Even if anoestrus occurs it is transient and disappears over the next few weeks as antibody titres decline. Since this is an active immunization, individual animal titre responses vary considerably and a declining titre curve accommodates those animals that may tend to have an over-vigorous response by allowing them to drop into a lower titre range or "window" during a 5-6 week joining period.

Further, it was considered an advantage to have titres declining sufficiently that values would be very low at the time of lambing. This ensures that antibody transfer of steroid specific antibodies in colostrum and milk to lambs in the first days of life is minimal. In any case, no adverse effects of such passive transfer have been detectable in the progeny (Wilson et al. 1983).

Such a system also ensures that ovulation rates have returned to near control values by the next season. This gives the farmer the freedom to choose whether or not to retreat his ewes. Retreatment with a single booster in each subsequent year results in an increase in ovulation rate very similar to that obtained in the initial year.

EXPERIMENTAL RESULTS

Ovulation responses, lambing percentages 1979 was the first year in which well-controlled responses were obtained with an androstenedione-7-HSA-DEAE-dextran system. Subsequently, procedures were refined and simplified over the 1980-1982 seasons (Cox et al. 1982), whilst consistent gains were maintained in ovulation rate, embryos carried and lambing percentages. It is a major advantage of the immunization procedure that ewes of moderate to low fecundity produce an increased number of twins but very few triplets. Some groups of animals have shown increases in lambs born over 4 consecutive seasons;
the first 3 years data have been reported (Cox et al. 1982).

**Basic treatment systems**  From the results obtained up to mid-1982, a basic protocol was defined giving optimal results with an androstenedione-7-HSA immunization. In this, 1.2 mg androstenedione-7-HSA in 5% DEAE-dextran/0.9% sodium chloride solution is administered in 1 ml at a single site subcutaneously in the upper third of the neck. Spacing between first and second treatments is 3-5 weeks. Synchronization of oestrus is not required. Rams are joined 2-3 weeks after the second injection or after the booster injection in subsequent years. If rams are joined earlier than 2 weeks, poor results are likely.

**Effect of nutrition and breed**  Immunization of animals on different planes of nutrition indicates that gains in ovulation rate due to immunization can be additional to gains from better nutrition (Smith et al. 1981; Croker et al. 1983). However, for Merino ewes in very poor condition and decreasing body weight - conditions under which immunization would not be desirable - one test has indicated poor responses. There may be poorer responses in Merinos in Western Australia (Croker et al. 1981; 1983), where joining usually has to be carried out after pastures have dried off and ewes are losing condition.

Much of the development work was done using Merino ewes (Cox et al. 1982). However, all breeds and crossbred ewes tested so far have responded well. Other than Merinos, those tested include Dorset Horn (Scaramuzzi et al. 1982), Romney and Coopworth (Smith et al. 1981), Perendale (Smith et al. 1982) and Border-Leicester x Merino ewes (Cox et al. 1983a). Further evaluations on-farm have extended the range (Geldard et al. 1983).

Extension of earlier concepts on steroid immunization and ovarian function has led to the development of a practical immunophysiological procedure for increasing fecundity in the sheep. The procedure is simple and the increase in fecundity is seen largely as twins with very few triplets. This work has culminated in extensive on-farm testing and the development of a commercial product.
production of first-cross mothers (Border Leicester x Merino ewes), Autumn prime lamb production and Spring prime lamb production.

MATERIALS AND METHODS

Field trials to determine the commercial potential of immunization to increase fecundity in ewe flocks have now been carried out on thirty-five commercial properties and five experimental farms throughout Australia and New Zealand. Results collected during 1982 and 1983 are presented here. All treated ewes were immunized against androstenedione using polyandroalbumin in DEAE-dextran according to the following protocol. A mob of ewes on each farm was randomly divided into two roughly equal groups and each animal was individually identified using a numbered ear tag. Ewes in one group were given two subcutaneous 2 ml injections of polyandro-albumin in adjuvant before the start of the joining period. The spacing between injections varied from 20 days to 35 days, with a mean of 24 days. The interval between the second injection and the start of joining varied from 0 to 46 days, with a mean of 15 days.

The following breeds and crossbreds were tested: Merino (8 farms), Polwarth (1 farm), Corriedale (5 farms), Romney (4 farms), Coopworth (3 farms), Perendale (1 farm), Border Leicester/Merino cross (13 farms), Border Leicester/Romney Cross (5 farms). Ages of trial ewes ranged from eighteen months (maidens) to 7 years. The average liveweight of ewes at the start of joining on each farm varied between 35 kg and 64 kg. Oestrous records were obtained on some farms by the use of ram harnesses and crayons (Sire-Sine). Of the 40 farms involved in this series of field trials, six commenced joining in Spring or early Summer (Oct-Dec) and the rest began joining in late Summer or Autumn (Feb-May). Ewes were subjected to real-time ultrasonic scanning by the method of Fowler & Wilkins (1982); the number of lambs born was estimated from the number of foetuses detected by this procedure.

RESULTS

Effect of spacing between injections on the number of lambs born

Varying the spacing between the two injections from 20 days to 35 days had no significant effect on the response to the immunogen with regard to the number of lambs born.

Effect of interval between the second injection and joining

(a) On number of lambs born As the interval between the booster dose and the start of the joining period was increased the response in the treated ewes improved (Fig. 1). At intervals of less than one week there was a small increase in the number of lambs born to the treated ewes (average 10%), which rose to increases of 29% and 28% respectively at intervals of between 2 and 3 weeks and between 3 and 4 weeks.

(b) On pregnancy rates As the interval between the booster dose and the start of joining increased there was a corresponding decrease in the percentage of non-pregnant (dry) ewes in the treated group compared to the controls (Fig. 2). At intervals of less than two weeks there was a significant increase in the proportion of dry ewes in treated groups, but at intervals over two weeks there was no significant difference between the groups.

(c) On conception rates As the interval between the booster dose and the start of joining increased, the ratio of treated to control ewes conceiving to the first cycle also increased (Fig. 3). At intervals of less than two weeks there was a significant depression of conception rate to the first cycle in
Fig. 1 The relationship between the boost-join interval (weeks) and the increased number of lambs born per 100 ewes joined in sheep immunized against androstenedione-7-HSA.

Fig. 2 The change in percentage of dry ewes (percent of treated ewes dry minus percent untreated ewes dry) at different spacings of the interval between booster immunizations and joining.
treated ewes compared with controls. At intervals of longer than two weeks there was no significant difference in first cycle conception rates between the two groups.

Effect of breed of ewe on response to immunization. The increase in the number of lambs born to treated ewes varied among the various breeds and crossbreds tested. Table 1 shows the response in each breed when the two injections of immunogen were given at the optimum time, ie three to four weeks between doses and two to three weeks between the second injection and the start of joining. Merinos responded to a lesser degree than Merino-crosses, Romneys and Romney-crosses. The greatest increase in the number of lambs born occurred in the New Zealand breeds of Romney, Coopworth and Romney crossbreds.

Effect of age of ewe on response to immunization. Immunization of all age groups from maidens (18 months) through to 7 year olds produced extra lambs born to the treated ewes (Fig. 4). No significant differences occurred between the age of groups tested. Maidens responded as well as the older ewes.

Effect of ewe weight at joining on response to immunization. As ewe weight at joining increased there was a corresponding increment in response to immunization (Fig. 5). The heavier the ewe at joining, the better the response to treatment. On average, ewes which weighed over 60 kg (empty bodyweight) at the start of the joining period produced an extra 40 lambs born per 100 ewes treated. At the other end of the scale, ewes which weighed less than 40 kg at the start of joining produced only 13 more lambs born per 100 ewes treated.

Effect of season of joining on response to immunization. Ewes treated and joined in Spring and early Summer responded as well to immunization against androstendione as ewes treated and joined in Autumn or late Summer (Table 2). As only Merinos and Border Leicester/Merino ewes were joined in Spring these breeds only were used to compare response in Autumn joining flocks.

<table>
<thead>
<tr>
<th>Breed</th>
<th>Lambs born per 100 ewes joined</th>
</tr>
</thead>
<tbody>
<tr>
<td>Merino</td>
<td>113</td>
</tr>
<tr>
<td>Crossbreeds</td>
<td>150</td>
</tr>
<tr>
<td>First-Cross (RT x WM)</td>
<td>147</td>
</tr>
<tr>
<td>NZ Breeds a</td>
<td>189</td>
</tr>
</tbody>
</table>

a Romney and Romney-Cross (eg Coopworth)

Effect of season of joining on response to immunization. Ewes treated and joined in Spring and early Summer responded as well to immunization against androstendione as ewes treated and joined in Autumn or late Summer (Table 2). As only Merinos and Border Leicester/Merino ewes were joined in Spring these breeds only were used to compare response in Autumn joining flocks.

<table>
<thead>
<tr>
<th>Season of joining</th>
<th>Lambs born per 100 ewes joined</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spring</td>
<td>125</td>
</tr>
<tr>
<td>Autumn</td>
<td>131</td>
</tr>
</tbody>
</table>

188
Fig. 3 The ratio of treated to untreated ewes that mated and conceived during the first oestrous cycle after joining as a function of the boost-join interval (weeks)

Fig. 4 The effect of the age of ewes at joining on the increased number of lambs born per 100 ewes joined in sheep immunized against androstenedione-7-HSA
DISCUSSION

Increasing the interval between the booster dose and the start of joining (a) improved the response in treated ewes with regard to the number of lambs born, (b) reduced the proportion of non-pregnant ewes in the treated group to the first cycle. From the results presented here, a minimum of two week (14 days) between the booster dose of polyandroalbumin and the commencement of the joining period is recommended. Ideally this interval should be 14-21 days. Crossbreds responded much better to the immunogen than did pure Merinos and for this reason one would expect a higher return on investment if a crossbred flock was treated. The fact that crossbreds are usually better mothers than Merinos indicates that the immunogen is more likely to produce a significant increase in weaning rates in crossbred flocks especially if the ewes are well-managed. Ewes of higher liveweight responded better to the immunogen than did ewes of poor body weight at the time of joining. This indicates that if producers are intending to use the immunization technique to improve lambing percentages they should endeavour to get their animals to the best possible liveweight at the start of joining. Alternatively it may be more economical to treat the top 50% of ewes on the basis of liveweight at joining.

It is important to remember that the extra lambs born to the treated group are mainly twins, with a few extra triplets (Geldard et al. 1983). Therefore, the immunized flock should be managed as one of high fecundity. This requires, perhaps, a higher standard of management on many farms with a possible reduction in stocking rate, better utilisation of pastures, provision of extra shelter during lambing and even the identification of multiple-bearing ewes before birth being aids which the farmer should consider for treated flocks. Survival to weaning of lambs from treated ewes has been shown (J.M. George, Pers. Comm.) to be the same as that of lambs born to control ewes. Survival rate studies of lambs born in the series of field trials described here will be reported later.
The application of this technique to an enterprise rearing first-cross ewes should be seriously considered where feed is available for the extra lambs which will be born to treated ewes. Following the recommended protocol Table 1 shows that a farmer immunizing Merino ewes should expect an average increase of 18 lambs per 100 ewes treated. With good management this should be reflected in an increased weaning of about 11%. Although these extra lambs will be nearly all twins, with good nutrition they should be indistinguishable from single-born lambs by 12-15 months of age. Prime lamb producers using first-cross mothers, Corriedales or Romney breeds should achieve an increase of about 25-40 lambs born per 100 ewes treated (Table 1). With good management of ewes and lambs an increase in weaning of between 15% and 30% can be expected. These extra lambs will be mainly twins with a few triplets and thus will require extra nutrition to reach the same marketable weight as single-born lambs. Despite this the good producer with pasture available should achieve a reasonable return on investment. Cox et al. (1982) have shown that a single booster injection is required in previously immunized animals to achieve the same response. Therefore it would appear to be more economical to treat the younger ewes in the breeding flock in the first year of an immunization schedule.

**CHANGES IN PITUITARY - OVARIAN FUNCTIONS IN EWES IMMUNE TO STEROID HORMONES**

R.J. SCARAMUZZI*

Immunization against the steroid hormone androstenedione using a covalently coupled steroid-protein conjugate leads to the formation of steroid binding antibodies which in turn leads to increases in ovulation rate (Scaramuzzi et al. 1977; Scaramuzzi 1979) and lambing percentages (Cox et al. 1982; Scaramuzzi et al. 1982). Immunity to androstenedione therefore increases lambing percentages primarily by increasing ovulation rate in the steroid-immune ewe. An understanding of the mechanisms by which Fecundin (polyandro albumin, Glaxo Australia Pty. Ltd Boronia Vic) increases lambing percentages will therefore involve study of the hormonal regulation of ovarian function.

**OVARIAN RESPONSES TO STEROID ANTIBODIES**

The ovaries of ewes immunized against androstenedione are heavier than ovaries from untreated ewes. The increased ovarian weight is due to both an increased number of corpora lutea (ovulations) and an increased weight of non-luteal tissue (Table 1). The individual corpora lutea of steroid-immune ewes also tend to be larger. The non-luteal tissue consists of ovarian follicles and the ovarian stroma and although we have no information of the relative weights of these two components we do know that the number of large Graafian follicles (>2 mm diameter) is increased in ewes immunized against androstenedione (Scaramuzzi and Hoskinson 1984). These changes can be readily observed in the hyper-stimulated animal, i.e., in ewes where the antibody titre to androstenedione is of the order of 1/25,000 or greater. The optimum titre for producing increases in lambing percentages is lower by approximately an order of magnitude and in such animals one might expect the changes described above to be less obvious and may even be absent.

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TABLE 1 The changes in the ovaries of ewes immune to androstenedione (Data adapted from Scaramuzzi and Hoskinson 1984)

<table>
<thead>
<tr>
<th>Type of ewe</th>
<th>Immune to androstenedione (Mean ± SE; n = 9)</th>
<th>Untreated controls (Mean ± SE; n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight of ovaries (g)</td>
<td>3.56 ± 0.55 **</td>
<td>1.63 ± 0.15</td>
</tr>
<tr>
<td>Weight of individual C.L. (g)</td>
<td>0.70 ± 0.01 *</td>
<td>0.57 ± 0.03</td>
</tr>
<tr>
<td>Weight of remainder (g)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(non-luteal tissue)</td>
<td>2.58 ± 0.52 *</td>
<td>1.23 ± 0.10</td>
</tr>
<tr>
<td>Ovulation rate</td>
<td>2.79 ± 0.46 *</td>
<td>1.44 ± 0.18</td>
</tr>
</tbody>
</table>

*P<0.05; **P<0.01

The ovary contains a large pool of non-growing primordial follicles and from this pool of follicles a number of follicles commence growth each day. The pattern of growth of these follicles in ewes immunized against androstenedione has also been studied (Table 2). The principal findings of this study were that steroid-immunity had very little observable effect on the

TABLE 2 The follicle population in ewes immunized against an androstenedione-protein conjugate (data adapted from Scaramuzzi and Hoskinson 1984)

<table>
<thead>
<tr>
<th>Type of ewe</th>
<th>Immune to androstenedione (Mean ± SE; n = 5)</th>
<th>Untreated controls (Mean ± SE; n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of healthy antral follicles</td>
<td>35.0 ± 10.2</td>
<td>45.0 ± 4.3</td>
</tr>
<tr>
<td>Number of early-atretic follicles</td>
<td>4.8 ± 1.9</td>
<td>6.0 ± 1.0</td>
</tr>
<tr>
<td>Number of follicles with advanced atresia</td>
<td>17.6 ± 5.2</td>
<td>26.4 ± 5.0</td>
</tr>
<tr>
<td>Number of large mature follicles</td>
<td>1.8 ± 0.2**</td>
<td>0.4 ± 0.2</td>
</tr>
<tr>
<td>Ovulation rate</td>
<td>2.0 ± 0.2*</td>
<td>1.4 ± 0.2</td>
</tr>
</tbody>
</table>

*P<0.05; **P<0.01

Follicle development is regulated by the gonadotrophins Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH) and the levels of these hormones in the peripheral blood of androstenedione-immune ewes have been
measured. The secretion of LH is known to be pulsatile and consequently the pulsatile pattern of LH release was also studied. Examples of the characteristic patterns of LH release during the luteal and follicular phases of the oestrous cycle in immunized and control ewes are shown in Fig. 1. These experiments lead us to the conclusion that the levels of LH are elevated throughout the oestrous cycle of the ewe immunized against androstenedione. Furthermore the increase in mean levels of LH is primarily due to an increase in the frequency of pulsatile LH release during the luteal phase of the oestrous cycle and to increases in the basal levels of LH during the follicular phase of the oestrous cycle (Scaramuzzi and Hoskinson 1984). The frequency of pulsatile LH release was not increased during the follicular phase of the oestrous cycle. However at the very high pulse frequencies normally seen during this phase of the oestrous cycle a 15 min sampling interval, as used in these experiments, may not be adequate to accurately measure pulse frequency during the follicular phase of the oestrous cycle. The results need to be confirmed by additional research using a more frequent sampling regime.

In contrast the levels of FSH measured during the oestrous cycle of ewes immunized against androstenedione are reported to be unchanged (Webb et al. 1984) or possibly even reduced (Martensz and Scaramuzzi 1979) when compared to levels in control ewes. It is possible that FSH levels have been effected by alterations in the levels of inhibin produced by the androstenedione-immune

Fig. 1 The levels of LH seen in untreated and androstenedione-immune (A4-7-HSA) ewes during the luteal or follicular phases of the oestrous cycle. (Taken from Scaramuzzi and Hoskinson 1984)
The reduction of biologically active androgen combined with an increased number of healthy follicles (the source of inhibin) may lead to increases in inhibin levels which then act to selectively suppress FSH levels. It is equally possible that the decreased levels of FSH are due to an increased output of oestradiol by the ovaries of immunized ewes which we know have increased numbers of large healthy follicles (Table 1).

The observed increases in ovulation rate in the presence of reduced levels or unchanged levels of FSH in androgen-immunized ewes can most probably be accounted for in the 'dominant follicle' theory of Baird et al. (1975). This theory holds that in monovular species such as sheep a single follicle assumes a position of dominance by suppressing levels of FSH to a point below which the final stages of growth and maturation of other large follicles is not possible. The dominant follicle however continues to develop because the pattern of dominance involves a reduced requirement for FSH by the dominant follicle. This reduced requirement is thought to arise from a local synergistic action of oestradiol produced by the granulosa of the dominant follicle which increases the responsiveness and sensitivity of that follicle to FSH.

Immunization against steroid hormones may act by preventing or modifying the normal expression of follicle dominance. Oestrogen immunity in ewes leads to increased levels of FSH (Scaramuzzi et al. 1980) and these increases alone could account for the increased ovulation rate seen in such sheep (Scaramuzzi 1976). Androgen-immunity in sheep however leads to decreases in circulating FSH and it is possible that the mechanism of action in this model involves an increased responsiveness to FSH modifying the pattern of follicle dominance such that the combined actions of two or more follicles is required to exert dominance.

Immunization of sheep using androstenedione protein conjugates then leads to the formation of antibodies to androstenedione. These antibodies may disrupt the normal feedback relationship between the ovary and the hypothalamo-pituitary axis and result in increased ovarian stimulation and increases in ovulation rate. It is suggested that this result is achieved by altering the normal expression of follicle dominance. These responses to immunization probably involve changes in ovarian sensitivity to gonadotrophin stimulation and/or inhibin production. Experiments to examine the consequences of steroid immunity on these areas of ovarian function are indicated from these results (Scaramuzzi and Hoskinson 1984).

HORMONE IMMUNONEUTRALIZATION AND THE MANIPULATION OF ANIMAL PRODUCTIVITY - A PERSPECTIVE

R.M. HOSKINSON* and P. DJURA*

EVOLUTION OF THE CONCEPT

Hormone immunoneutralization (HIN) means the in vivo neutralization of the biological activity of an endogeneous hormone by antibody. This is a specific example of the concept of autoimmunity which, in the endocrine field, dates back to the 1930's and studies of autoimmunity against the then known protein hormones (Collip et al. 1940). Landsteiner's (1934) hapten principle was developed at that time and brought the immunological study of all hormones within reach - whether or not they were inherently immunogenic.
The advent of radioimmunoassay procedures for hormone assay stimulated much interest in methodologies for raising antisera with specific binding characteristics for many hormones. However biologists were slow to take an interest in the biological consequences of hormone-immunity in animals, and slower still to recognise that productivity advantages might be derived in this way. Nevertheless it is now widely recognised that HIN is an extremely subtle and specific method for perturbing the endocrine system. Application of this technique has the potential to stimulate productivity wherever endocrine factors suppress the full expression of the genetic potential of an animal.

This article briefly highlights selected advances in the life sciences that are likely to influence the growth of hormone immunoneutralization research; it deals with a burgeoning array of experimental techniques, with certain trends in endocrinology and with a number of current studies to enhance animal productivity by HIN methods other than those based on the sex steroids.

AN EXPANDING ARRAY OF EXPERIMENTAL TECHNIQUES

HIN is developing as a hybrid discipline. Because it is concerned with provoking an immune response to small non-immunogenic molecules (increasingly, peptides) HIN technology depends upon advances in chemistry and immunology for improvements in its own methodologies.

In chemistry, advances in peptide synthesis (Li et al. 1983), improved methods for synthesising protein-peptide conjugates (Atassi et al. 1981) and preparative procedures for chemically modified immunogenic carbohydrates (Inman 1975) all provide for increased control of the composition of synthetic immunogens.

Immunological methods for stimulating the antibody response to immunogen have been attended by many developments (Borek 1977). Characterization of responses potentiated by water-soluble polyelectrolyte adjuvants has been of great value where a relatively acute antibody response is sought (Houston 1976). Painstaking elaboration of the water soluble extracts of immunostimulating mycobacteria culminated in the characterization, synthesis and commercial supply of muramyl dipeptide (MDP). There is a large literature concerned with the adjuvant properties of MDP when used in vaccines in admixture with immunogen (Chedid et al. 1978), however a fascinating development has been the use of the adjuvant in chemical combination with antigen. One report (Audibert et al. 1982) demonstrates the feasibility of developing totally synthetic vaccines which, in the HIN field, would consist of synthetic polypeptide immunogen to which would be chemically linked both target hormone and immunoadjuvant. Such vaccines should permit an increased control of the immune and physiological responses to hormone-immunity in animals.

New insights into the nature of antigenic determinants have been gained in recent times. Classical conformational determinants (Crumpton 1974) are tertiary structures formed by folding and looping of the peptide chain, that are confined to unique areas on the molecular surface. The immune response is usually directed against a small number of such determinants. Recently it has been shown that chemically synthesized peptides corresponding to virtually any amino acid sequence occurring on the surface of a protein can elicit antibodies that are reactive with the native molecule (Lerner 1982). The significance of this development to HIN research lies principally with peptide and protein hormones where it may be possible for fragments rather than entire molecules to provide the desired immunoneutralization.
A significant achievement in endocrinology has been the extensive elaboration of many endocrine control mechanisms; these provide a framework for conceptualizing likely interactions between endocrine function and the expression of a productivity character. Two key elements are: (i) the action of hormone-releasing and hormone release-inhibiting factors on pituitary function and (ii) the modulating effects on homeostatic equilibria of positive and negative hormone feedback mechanisms.

The discovery of a host of peptide or protein growth factors, gastrointestinal hormones and endogenous opiates appears to offer considerable potential for HIN techniques both for the elucidation of endocrine control mechanisms and for opportunities to manipulate animal productivity.

Still in its infancy is the study of anti-hormone-receptor antibody (Strosberg et al. 1981; Schreiber et al. 1981). Conceptually, an ability to affect events at the hormone-receptor site could readily complement antibody effects on the hormone itself. Amongst other effects anti-hormone-receptor antibodies have (i) blocked both the binding and biological activity of the hormone; (ii) mimicked the physiological action of the hormone without interfering with hormone binding. The multiplicity of effects is probably a consequence of the natural antibody heterogeneity in the immune response; detailed explanations of them will probably require studies with monoclonal antibodies.

**Attempts to apply HIN principles to enhance animal productivity**

Early research sought to obviate the anti-reproductive effects of ingested phyto-oestrogens in the sheep. Equol-immunity did not affect the expression of oestrous behaviour (Cox et al. 1976) and, in an acute study, it diminished substantially the oestrogenic response of uteri to infused equol (Cox et al. 1983). For chronic protection of the ewe by equol-immunity the central problem will be one of stoichiometry - whether it will be possible for the immune system to produce sufficient antibody daily to neutralize the quantity of toxin entering the blood.

Of importance to reproduction research is the immunoneutralization of inhibin, the gonadal peptide that inhibits the synthesis and release of follicle stimulating hormone. Using steroid-free extracts of bovine follicular fluid O’Shea et al. (1982) made the ewe immune to bovine inhibin and observed significant increases in ovulation rate. Inhibin is currently the subject of extensive structural studies. Elucidation of its amino acid sequence and concomitant improvements in the nature of the inhibin antigens will facilitate this alternative approach to the enhancement of fecundity (prolificacy).

Immunization of the male pig against 5α-androst-16-en-3-one failed to inhibit the partition of steroid between plasma and body tissue and thus could not be used as a prophylactic against “boar taint” (Shenoy et al. 1982). Here was an immunological attempt to improve the quality of a meat product in order to take economic advantage from the greater growth rate of the entire animal, compared to a castrate.

Immunity to luteinizing hormone releasing hormone (LHRH) in ram lambs and bull calves diminished testicular size in these animals as well as plasma testosterone concentrations. These findings demonstrated the potential of LHRH immunoneutralization as an alternative to castration (Jeffcoate et al. 1982). Nevertheless a related study (Schanbacher 1982) on young ram lambs confirmed
that LHRH-immunity and testosterone-immunity blocked the growth attributes characteristic of intact ram lambs. Robertson et al. (1982) proposed temporary LHRH immunity in bulls for beef producers who might wish to rear entire bulls to slaughter. In the immunocastrates they found reduced libido and semen production, docile behaviour and a superiority in productivity traits compared to steers.

Thyrotropin releasing hormone immunity has been the subject of a longitudinal study in the ewe (Fraser and McNeilly 1982). While good antibody responses were found and minor effects on prolactin, T₃ and T₄ were noted the immunity had no apparent effect on the time of onset of the breeding season, estrous cycle or on pregnancy. Ewe body weights were unaffected by the treatment but wool production was depressed significantly.

Immunoneutralization of the endogenous inhibitor of growth hormone (GH) release, somatostatin, has created considerable interest. One report of somatostatin-immune lambs demonstrated an increase in serum GH concentrations but a failure to increase growth (Varner et al. 1980). Others failed to detect effects on basal GH levels but achieved significant increases in growth rates of the immune lambs (Spencer and Williamson 1981; Spencer and Garssen 1983). An intriguing related observation (Muir et al. 1983) is that lambs given daily doses of ovine growth hormone did not change their average daily body weight gain or feed intake. This suggests that growth-promoting effects of somatostatin immunity may be mediated by endocrine pathways not involving growth hormone but, conceivably, by way of effects on other hormones known to be inhibited by somatostatin (insulin, glucagon or thyroid stimulating hormone).

To conclude, these data suggest that HIN research which now has a substantial basis in science also has considerable potential to be among the biotechnologies that will be used in the future to stimulate animal productivity.

REFERENCES