DEPARTMENT OF AGRICULTURE AND RURAL AFFAIRS
VICTORIA

DEVELOPMENT AND EVALUATION OF NEW CHEAP AND EFFECTIVE HORMONE DELIVERY SYSTEMS TO CONTROL BREEDING

FINAL REPORT

PROJECT DAV 22S

Sponsored by

AUSTRALIAN MEAT AND LIVESTOCK RESEARCH
AND DEVELOPMENT CORPORATION

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AUSTRALIAN MEAT AND LIVESTOCK RESEARCH & DEVELOPMENT CORPORATION

FINAL REPORT

1.	Name of Organization:	Department of Agriculture, Victoria.
2.	Project Number:	DAV 22S
3.	Title of Project:	Development and evaluation of new cheap and effective hormone delivery systems to control breeding.
4.	Location:	Animal Research Institute, Werribee.
5.	Division, Department or Section:	Animal Research Division, Physiology Section, Werribee.
6.	Name and Position of Project Supervisor:	A.H. Williams, B. Agr. Sc., Livestock Research Officer.
7.	Research Staff:	L.D. Staples, M. Agr. Sc., Ph.D. Physiology. I.J. Clarke, M. Agr. Sc., Ph.D. NH & MRC, Research Fellow R. Oppenheim, B. Sc., Ph.D, FRACI Senior Lecturer, Victorian College of Pharmacy

8. Project Description:

AIM: The primary objectives of this project were specifically limited to the development and testing of prototype subcutaneous implants to provide a controlled delivery of Gonadotrophin Releasing Hormone (GnRH) for the purpose of inducing ovulation in anoestrous ewes. The project was originally foreshadowed as a three year project with a schedule of target objectives as follows:-

Phase 1:- Preparation of various matrix formulations to obtain a controlled delivery of GnRH.

Phase 2:- Testing of implants in preliminary trials to examine effects on ovulation and luteinizing hormone release.

Phase 3:- Preliminary field testing and optimization of treatment regimes.

PROGRESS: This is the second and final report on this project which was regarded as a small seed project in which a prototype implant was to be developed to induce ovulation in anoestrous ewes based on known parameters ovulations induced by infused or injected GnRH. This objective has now been achieved successfully. Any subsequent large scale development was to proceed only if adequate progress was made during the initial project and in particular if the feasibility of an effective sustained release implant for GnRH could be demonstrated.

The experimental approach was a collaborative effort with three main areas of activity as follows:

1) implant development primarily at the Victorian College of Pharmacy.

2) in vivo testing of implants in sheep primarily at the Dept. of Agriculture and Rural Affairs

and 3) assay of plasma samples for various steroid and protein hormones primarily at Prince Henry's Hospital.

9. Funds Provided - other sources of funding:

In 1984/85 the project was supported by external funding of $15,883 from AMLRDC. A further $12,700 was committed in 1985/86 from AMLRDC funds. In June 1985 Glaxo Aust. Ltd. expressed interest in the project and contributed a further $15,300 primarily to speed development of implants during the period from June to Dec. 1985. (Details of arrangements with Glaxo have been reported previously to AMLRDC.) With this additional assistance, progress in achieving objectives was accelerated so that this final report can be submitted one year ahead of schedule.

10. Experimental Results

Summary: Several uncoated implants made of various matrices were treated for delivery of GnRH. This approach proved unsuccessful. Coated implants were then tested using rate-limiting membranes of cellulose acetate phthalate. While this formulation provided adequate delivery in vitro, the implants were unstable in vivo.

The coating and core were then redesigned and several combinations were tested in vivo using oestrogen-treated speyed ewes and entire ewes.

The final implant coated with an acrylic ester has resulted in a satisfactory release profile of GnRH suitable LH profiles in spayed ewes and induces ovulation in 47-84% to anoestrous ewes.

(a) Uncoated Implant Studies. The first approach to implant development was to obtain a controlled delivery of GnRH from a biocompatible implant from which hormone release was controlled by the combined effects of drug diffusion and core matrix erosion.

Parameters investigated included:-

1) The type and relative proportions of excipient materials in the core. Excipients tested were Lactose, Emdex and Calcium Phosphate and various mixtures thereof.
2) The concentration of the hydrophobic lubricant magnesium stearate.
3) The concentration of water soluble binding agent for optimal wet granulation of excipients.
4) The hardness of the compressed implant, and
5) The degree of mixing of granules with Mg stearate.

Implants were assessed in vitro for dissolution and physical stability in a serum/saline suspension and in vivo at various times after subcutaneous implantation.

Critical factors for stability of the implant core were found to be the relative proportions of excipient materials and the concentration of magnesium stearate.

Implants consisting of Lactose with 5% magnesium stearate and containing 30 ug GnRH were tested for GnRH release in vitro and for induction of Luteinizing hormone (LH) release in ovariectomized ewes. GnRH release in vitro was rapid with maximum values GnRH release observed within 3 hr. These implants also induced an LH release in vivo within 1 hr of implantation, LH peak values in response to these implants were more than 200% higher than those achieved with GnRH releasing osmotic minipumps which are satisfactory for induction of ovulatory responses.

The proportion of insoluble components (calcium phosphate) was increased in an effort to obtain a more gradual release of GnRH, but again these implants were shown to induce a rapid short term LH release.

Calcium phosphate implants containing 30 ug or 10 ug of GnRH were also tested for induction of ovulation and LH responses in entire progesterone primed anoestrous BL x M ewes. Plasma LH levels were dose related (up to 80 ng/ml for 30 ug implants and up to 25 ng/ml for 10 ug implants) but LH levels declined to or below pretreatment levels within 6 h of implementation. These uncoated implants did not induce ovulations.

(b) Coated implant Studies. Release of GnRH from uncoated implants was too great and of insufficient duration to permit proper follicular development and ovulation. Coated implants were therefore developed with a view to achieving a controlled release of GnRH via a semipermeable rate limiting membrane. The first membrane tested consisted of the enteric coating material cellulose acetate phthalate, the porosity of which was varied by changing the content of hydrophilic polyethylene glycol in the coating formulation.

Excellent delivery characteristics were observed in vitro for GnRH and for water soluble dye markers but in vivo studies showed these coatings to be unstable at physiological pH due to pH dependent ionisation of carboxyl groups and consequent hydration of the coating film. Induced LH releases were therefore rapid and transient in steroid treated spayed ewes and none of these implants induced significant numbers of anoestrous entire ewes to ovulate. Full details of experimental results from the first year of this project were given in the first progress report.

(c) Revised coating procedures and design modifications.
Following support from Glaxo a graduate research assistant (committed to this project) was appointed at the Victorian College of Pharmacy. This enabled more rapid progress to be achieved and resulted in the development of an improved coating. Also modifications of size and shape were made to the implant core to obtain an implant suitable for practical administration to ewes in the field.

New implant cores were prepared containing 12 or 24 ug quantities of GnRH with exipients of normal or micronized calcium phosphate and coated with various thicknesses of a commercially available acrylic ester resin "Eudragit E30D". Comparison was also made between gum acacia and polyvinylpyrolidone (PVP) as a granule binding agent. The Endragit coating is a neutral copolymer of poly(meth) acrylic acid esters. A modified pan coating procedure was developed at the Victorian College of Pharmacy to enable small batch production. Coating parameters optimized included pan temperature, air flow, air temperature and humidity, coating spray rates, tumble speed, pan loading and film solvent type and concentration.

Implants were assessed in vitro for release of GnRH, salicylic acid and iodinated peptides and proteins of various molecular weights. Promising implants were also assessed in vivo for their effects on LH release in oestrogen treated, ovariectomized ewes and for induction of ovulation in anoestrous entire ewes. Responses to implants were compared to those achieved with GnRH-releasing osmotic mini pumps. Full details of these experiments are contained in the second progress report (Attachment 1).

Five trials were conducted at Werribee and are summarized as follows:-

Trial 85/01 Assessment of implant core formulation parameters on GnRH-induced LH release in oestrogen-treated spayed ewes.

20 E_2-treated spayed ewes (5 groups of 4) were implanted with blank implants, osmotic mini pumps (250 ng GnRH/h) or implants containing 12 ug GnRH. Cores based on calcium phosphate or Lenphos

excipient material and coated with Eudragit E30D (3.5% w/w) were compared with uncoated calcium phosphate implants.

Major conclusions (see also attachment 1 for detailed results).

* the E_2 treated spayed ewe model provided sensitive <u>in vivo</u> test for GnRH - induced LH release.

* uncoated implants, from which GnRH release was by combined effects of matrix erosion and diffusion dumped GnRH to soon and too fast.

* LH responses to GnRH from Lenphos cores were of slightly reduced "dumping effect" compared with calcium phosphate cores.

* A 3.5% coating of Eudragit E30D substantially improved LH response characteristics by delaying time of peak response, reducing total LH release and lowering peak LH levels.

<u>Trial 85/02</u>. Assessment of the effects of increasing implant coating thickness on the induction of LH release in oestrogen-treated spayed ewes.

Twenty eight oestrogen treated spayed ewes received blank implants (-ve control), osmotic mini pumps (250 ng/h GnRH, +ve control) or Lenphos implants containing 12 ug GnRH and coated with 0, 2%, 3%, 4% or 6% w/w Eudragit E30D.

Major conclusions (see also attachment 1 for details).

* osmotic minipumps caused a sustained elevation of LH commencing 4-10 h after implantion and lasting for 8-20 h. Mean total release (as determined by area under LH vs time curve) of LH was 202 ± 62 ngh/ml and peak LH concentrations of 31 ± 10 ng/ml were observed 8 ± 1 h after implantation.

* uncoated implants caused a rapid LH surge commencing 4-5 h after implantation and lasting 7-10 h. Total LH release (390 ± 99 ngh/ml) was almost double that observed with MOP's and peak values of 107 ± 24 ng/ml were observed only 4 ± 0.5 h after implantation.

* Increasing the coating of Eudragit progressively delayed the time taken to reach peak LH, the magnitude of the total LH release and the peak LH concentration.

* From these results it was predicted that Lenphos implants containing 12-24 ug GnRH and coated with 1-4% Eudragit E30D would mimic the <u>in vivo</u> responses achieved with osmotic minipumps.

<u>Trial 85/03</u> The effect of changing coating thickness on PVP granulated calcium phosphate cores was also investigated <u>in vivo</u> in oestrogen treated spayed ewes.

Trial design and results were substantially the same as those described previously for the coated Lenphos cores (85/03 see also details in Attachment 1).

No substantial advantage was found to justify further use of the calcium phosphate/PVP cores and, in view of the concern about the use of PVP in therapeutics for cattle (occasional induction of anaphylactic responses) it was decided to pursue only the Lenphos cores.

Trial 85/04. Induction of ovulation and oestrous in entire anoestrous Corriedale ewes.

300 Corriedale ewes were monitored for oestrous cyclicity using harnessed vasectomized rams. When spontaneous cyclicity was less than 10% in early November all ewes underwent endoscopy and 200 anovulatory ewes were selected for the trial. These ewes were treated with intravaginal progestagen sponge pessaries for 10 days prior to implantation. At sponge withdrawal, groups of 19 or 20 ewes received subcutaneous implants containing approximately 12 or 24 ug GnRH and coated with 1.25, 2 or 4% Eudragit. Coated and uncoated blank implants served as negative controls while osmotic minipumps were to serve as positive controls. Uncoated 12 ug GnRH implants were also tested as negative controls as these had been previously shown to induce premature LH releases and previous trials with uncoated implants had failed to induce ovulation.

All ewes were observed twice daily for oestrus and underwent endoscopy 10 days after implantation. Jugular venous blood samples were taken for progesterone assay 7, 11 and 14 days after implantation to obtain a quantitative measure of the adequacy of the luteal phases in those ewes which had corpora lutea.

Major conclusions:- (full details are given in Attachment 1).

* oestrous was observed in 15 - 52.6% of the ewes treated with coated implants compared to only 2.63% of ewes showing oestrous in the combined control groups and 5% in the group which received uncoated 12 ug GnRH implants.

* Of the 39 ewes which did exhibit oestrous most (27) did so within 48-72 h after sponge withdrawal and implantation.

* highest incidence of oestrous 9/19 (52.6%) was seen in those ewes which received 24 ug GnRH implants coated with 4% w/w Eudragit.

* Similarly the incidence of ovulation was substantially improved in the groups treated with coated implants (20 - 84.2%) compared with the combined controls (overall 7.9% ovulating). Ovulatory responses for the 24 ug implants appeared to improve as the coating thickness was increased.

* highest incidence of ovulation 16/19 (84.2%) was seen in the group treated with 24 ug GnRH implants coated with 4% Eudragit E30D.

* Luteal phases were of normal duration.

* No evidence was found for supernormal ovulation rates.

* Only 52.6% of the 16 ewes ovulating after treatment with the 24 ug 4% coated implant also showed oestrous.

Trial 85/05. A second experiment was conducted in anoestrous entire Corriedale ewes to confirm the effectiveness of the 24 ug GnRH, 4% Lenphos implants. This experiment was conducted in late December, seven weeks after the initial trial.

The experimental design was similar to that used previously (85/04) except that blood samples were not collected and, once oestrus was observed with vasectomized rams the oestrous ewes were immediately penned with entire rams in an attempt to achieve fertile matings.

Progestagen pessaries were inserted in 34 entire anoestrous ewes for 7 days and, at sponge withdrawal, 17 ewes remained untreated and 17 ewes received Lenphos implants containing 24 ug GnRH and coated with 4% Eudragit.

Major conclusions:-

* 8/17 (47%) of treated ewes ovulated compared with no control ewes ovulating.

* 8/17 (47%) of treated ewes mated but only 5 of these also ovulated.

* no control ewes mated during the observation period.

From trials 85/04 and 85/05 it is clear that a coated implant containing GnRH has been shown to induce ovulatory responses in 47 - 84% of anoestrous Corriedale ewes. About half of the animals treated also showed oestrus. The implant is biocompatible and is designed to permit simple administration in the field using a multidose implant gun. The design, and hormone load are such as to permit scale up production at a unit price sufficiently low to achieve acceptance in the market place.

Commercial Application from this research project

a) Patent Application

The research results have demonstrated that a controlled delivery of GnRH *in vivo* can be achieved using a biocompatible implant. The implant has been designed for practical manufacture and easy administration in the field. The quantity of hormone required is low and the hormone is identical to the natural GnRH produced by the animals. The project has therefore developed a practical, cheap and effective therapy to induce ovulation in anoestrous ewes. No product registration difficulties are envisaged although stability testing is still required.

Advice has been sought by independent patent Attorneys (Davies and Collison) as to the novelty of the implant development. Dr. Corbett of Davies and Collison has advised (Attachment 2) that two patentable opportunities arise from the work.

(i) A specific patent claiming invention of an implant to induce ovulation by delivery of GnRH.

(ii) A more general claim of the general implant design for the controlled delivery of a variety of hormones, drugs and other therapeutic agents.

Draft provisional patent specifications are being prepared.

b) Ownership

It has been suggested that, on the basis of all inputs, that the proportion of ownership of the invention attributable to the collaborating organizations (as a group) would be distributed in a ratio of 40 DARA : 30 PHH : 30 VCP

Proportioning of ownership between the collaborating organizations (as a group) and AMLRDC has not yet been determined.

Glaxo (Aust.) have expressed an interest in the project and commitment has been made between the collaborating group and Glaxo that first option to develop a product from this work should be offered to Glaxo. The terms and conditions of such licence agreement would be the subject of discussions between all interested parties including AMLRDC. Glaxo do not own any part of the patent(s) but are privy to current data under cover of a confidentiality agreement.

c) Further work

Given the potential of this research for generating a marketable product for use in Australia and overseas it is considered that an expanded experimental programme is required to address the following issues:-

(1) improvement in ovulatory response through further refinements in the implant.

(2) detailed description of the endocrine events following insertion of the implant.

(3) improvement in the expression of oestrus in ovulating animals and demonstrations of the fertility of induced ovulations.

(4) testing the effectiveness of the implants at different times of the anoestrous period and for different breeds.

(5) development of optimum treatment strategies for use in conjunction with artificial insemination.

(6) examination of the influence of rams on responsiveness to the implant.

A full proposal for a new project to encompass these studies is to be submitted to AMLRDC for consideration for sponsorship commencing in 1986/87.

CONTROLLED DELIVERY FORMS OF GnRH TO INDUCE OVULATION IN ANOESTROUS EWES

Collaborative project involving

Department of Agriculture and Rural Affairs, Victoria

Medical Research Centre, Prince Henry's Hospital and

Pharmaceuticals Division of Victorian College of Pharmacy

Sponsored by

Australian Meat and Livestock Research and Development Corporation

and

Glaxo Australia Ltd.

ATTACHMENT II

PATENTING ADVICE

December - 1985

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19th February, 1986.

Dr. Linton D. Staples,
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Dear Linton,

re Controlled Delivery Forms of GnRH

I refer to the various discussions we have had on the above matter, together with representatives of Prince Henry's Hospital and the Victorian College of Pharmacy.

I have reviewed the information provided by you, including the second progress report of 2nd December, 1985, and now provide the following advice as requested.

Generally, it appears that the broad concept of using GnRH to induce ovulation in anoestrous ewes is known and published. I also understand that the concept of using an implantable device to provide controlled delivery of hormones or other biologically active substances is known but that, to date, no satisfactory delivery system has been found which will provide the required profile of administration for GnRH.

The Department, in conjunction with Prince Henry's Hospital and the Victorian College of Pharmacy, has developed specific formulations for implants which will provide the necessary release characteristics for GnRH. Laboratory and field trial data provided by you indicates that the new formulation will provide the required delivery rates and profile and that in the field it is capable of producing the desired control of ovulation, at least to a statistically significant extent.

On this basis, I consider that there is patentable subject matter in the method of delivery developed by you and your collaborators and also for the formulations described in your report.

Cont...

Cont...

Preliminary searches conducted in computer data-bases to which we have access indicate that the proposed formulation is novel and, accordingly, on present indications, it would appear that patent protection could be obtained for the implant formulations and the method of delivery.

I also note that the formulations and delivery methods developed have already been demonstrated to have potentially broader applications and may be used for the delivery of hormones other than GnRH and possibly other pharmaceutically active substances. These further applications thus also seem to involve patentable subject matter and, providing reasonable supporting data can be obtained, I would expect that patent protection could be obtained for these broader applications.

Finally, I confirm that I am presently drafting specifications to cover both the narrow and broad aspects of the formulations and methods of delivery discussed above and I hope to forward the draft specifications to you in the near future. In the meantime, please advise whether you require any further advice or information.

Yours sincerely,

T.G. Corbett