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Review of the alternatives to castration and spaying of ruminants

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Abstract

Surgical castration is a long entrenched traditional husbandry intervention for cattle, sheep and goats. Surgical spaying only occurs in cattle. Finding alternatives and thereafter doing things differently on farm, by conviction, will not come "naturally" to production livestock owners/managers. However neither did accepting the polled condition (replacing horns) reality for the red meat industry during the 20th Century!!!

This review, in acknowledging this background reality, has recommended transitional arrangements eg channel webbing, and husbandry adaptation eg grow out and marketing of entire young male progeny, to allow industry to more easily adapt to and manage that external change whilst the R&D pipeline delivers! This will allow incremental progress towards the necessary social (community) contract goal of replacement of these surgical interventions in older animals, whilst allowing their retention in the young – <3-6 months being the watershed age in current welfare negotiations.

The very fact of the current welfare standards and guidelines negotiations and industry participation therein, foreshadows a changing future reality moving inexorably towards accommodating some of the current and likely future animal welfare community expectations. In this pursuit, necessary change and productivity improvement must be locked together, so that industry benefits most from the external, even unwelcome, pressures for that necessary change. The breeding of bare breech sheep is the "mulesing debate" equivalent scenario.

The capacity to research and develop a preferred animal welfare future for the industry over the next 5 years led to this review in 2013. The aim of MLA was to refine and develop the R&D program already underway to equip industry with viable alternatives that could effectively replace spaying and castration over time.

These two procedures (castration and spaying) deliver contraception/sterility to the majority of males (eg steers) and selective sterility/contraception to cull heifers/cows being excluded from the breeding herd for genetic selection, age culling and/or economic (cash flow) reasons. The aim of any replacement alternatives should encompass the same goals AND enhance productivity through improved herd performance, seasonal management control/efficiency or cost savings eg labour.

The project brief was to review the scientific literature together with the current portfolio of relevant projects and to make recommendations identifying promising areas for the R&D program through to 2017, cognisant of identifiable risks for success and failure.

Following an initial half-day issues scoping meeting with key current researchers in Brisbane, the literature review process took a blank page starting point and developed a broad key word based search strategy, largely directed at contraception in man and animals. Relevant reviews were identified and key topic areas defined and explored. A broad sweep of technologies were investigated including both "central", eg GnRH, and "peripheral" gonad targeted mechanisms eg zona pellucida vaccines.

Overall the conclusion was reached that the science is "looking good" for contraceptive reproductive control given multiple global drivers for research, including human population control, wildlife conservation and management, urban stray pet/pest control AND extensive livestock management. These diverse drivers currently engage a highly competitive and targeted global workforce of scientists and researchers both academically and commercially focused. This is evident in the review content.

However, focused additional R&D effort in the less emphasised field of production animals is certainly indicated, to exploit known basic scientific technologies, refine these approaches and deliver novel applications and application studies critical to final commercial success for livestock producers.

This R&D effort and emphasis is particularly indicated, especially as species driven variations in response are prominent within the review, and must be defined, addressed and/or exploited for production livestock.

An incremental project/technology based approach is recommended together with collaborative resourcing (academia with commerce and industry), flexible and multi-pronged program areas of investment and a focus towards low cost/easy to use final outcomes and product specification eg one NOT two dose GnRH vaccine technology.

The need for new basic science in production animals is also clearly evident given early stage (last 10 years in laboratory animals) discoveries eg kisspeptin peptides. This highlights the need for a deliberate R&D pipeline with a realistic time horizon (say 10+ years) and a determined, persevering policy for investment governance and approvals. Replacement of surgical interventions can and will come but it will not come without sustained effort from all stakeholders.

Human population control and contraception is a key resource for new ideas and science which can drive the reproduction prevention focus. Industry should seek collaborations with key institutions/researchers such as Aitken and McLaughlin et al at the University of Newcastle, NSW or current USA and Chinese published academics cited in the review.

Recommendations in the report cover applied commercial R&D together with more strategic and basic research strategies. The need for a broad portfolio of investments, across most scientific technology approaches, is evident, particularly as commercial outcomes to meet industry specification have already proven difficult to achieve.

Priority R&D areas are identified and justified. These include GnRH chemical agonists for application in cows and heifers followed by long-acting single dose GnRH immune-contraceptive vaccines of high efficacy. This latter specification may demand innovative alternatives eg DNA vaccine approach, to achieve.

The report recommends re-engagement with the developers in the USA in the field of GnRH toxin conjugates which have potential to ablate pituitary GnRH secretion of this hormone, centrally disrupting reproduction in both males and females. Again application studies in large production animals, initially rams, are the necessary next proof of principle step(s).

Finally for “central” mechanisms the experimental biology of kisspeptins seems a new and warranted basic R&D exploration, again initially in sheep where some bio-activities have already been defined/published and which indicate a potential to allow effective contraception.

In contrast to contraception delivered through higher “central” mechanisms, there is significant published science dealing with technologies targeting gonads and other “peripheral” reproductive organs eg uterine endometrium. These are considered for both male and female production animals in the review.

The most widely explored ovarian target is the female egg zona pellucida (ZP) glycoprotein complex. Multiple species have been targeted over 30+ years with considerable success but a porcine ZP antigen vaccine for domestic cattle remains a developmental application challenge.

Currently Queensland University animal scientists are conducting proof of principle studies and when that is completed the greater challenge of industry application research, linked with commercialisation, will need to be addressed. MLA levy funding and/or MLA Donor Company support of such commercial investment is encouraged.

In contrast more basic research should be contracted around the anti-implantation (uterine endometrium as target) approach and, more particularly, the broad gonadal germ cell contraceptive strategy developed in laboratory animals by Professor R.J (John) Aitken at

Newcastle (NSW) University. An opportunity for future collaboration based on this science is outlined in the recommendations and should be pursued in a timely way in 2014 with CSIRO.

The focus on male cattle is warranted as, apart from the GnRH vaccine approach, there are no other advanced male non-hormonal contraceptive technologies which commercially could be simply adapted for livestock. This also reflects the paucity of human male contraceptive R&D in general over the years, which is only now being redressed eg Li-Hua Yang et al (2011).

This review has a final dilemma of multiple competing technological choices, many of which have not even been adequately investigated in commercial livestock. This presents an ongoing, challenging and complex decision making paradigm which will need close on-going R&D managerial oversight by MLA.

Attention must first be paid to specified delivery outcomes at industry herd/flock level and then to the likely product/service outcome, it's scientific and commercial viability, including efficacy and likely cost/benefits for both suppliers and industry users. A comprehensive win-win scenario alone will suffice!!!

MLA (on behalf of industry) has a vital and pivotal role to broker and facilitate the factual, critical science investment and the related, necessary, frank discussions with commerce and industry to define and follow key critical developmental pathway(s) to success in the longer term.

Welfare (including castration and spaying) replacement technologies MUST emerge to facilitate better industry productivity, including labour productivity, NOT to primarily placate an animal welfare or other activist position. Better animal welfare and associated health outcomes, with concurrent improved productivity – both animal and animal management - will then recommend and sell themselves to MLA members.

Executive Summary

This report expresses a positive perspective that continuing scientific advances in reproductive understanding, and the broad capacity to thereby prospectively manage and to control reproductive outcomes in the future, is “looking good” in animals and mankind. This is concluded from the review which reveals more than a century of accumulated knowledge in the field of contraception, much of which is unfamiliar or unknown to many in agricultural livestock production.

The disparate but key research drivers emanate from clear needs for human population control and family level reproductive control, followed closely by nature conservation/wildlife management and urban “companion” animal issues eg stray dog and cat management and more recently, the welfare of managed animals for food production. All these diverse priorities for research (past and present) are clearly reflected in the broad contraception literature reviewed for the project.

However there is the risk of individual science inspired technologies under-performing in production animals, especially if insufficient developmental research is committed over many years to precisely define, understand and exploit the potential performance outcomes in each production species. Indeed the significance of species variation is high-lighted continually eg with zona pellucida and GnRH vaccines, in this scientific field. Thus application studies (species x gender x technology) become critical to success.

The report recommends activities for setting and contracting, practical, achievable and incremental research and development projects/goals within a longer-term research horizon. The strategic target of permanent sterilization (comparable to the current outcomes from both surgical castration and spaying) should be preceded by incremental advances in longer lasting contraception, moving from say two shot contraceptive vaccines to one shot extended duration technology. This step alone has proven to be a major novel research challenge, which is still incomplete for most species including cattle, sheep and goats. It has however been made to work for free-ranging white tailed deer in eastern USA.

Further, multi-pronged funding flexibility is needed to research diverse ideas and approaches, both practical and theoretical, which might work only in one livestock species or sex. This is exemplified by the recommendation for a project approach to channel spaying of female cattle or the investigation of GnRH agonist technology, specifically for rams which are to be culled from seed stock production systems but which are too large as sub-adults for routine, welfare approved manual castration procedures by non-veterinarians.

There is also a very real need for a broad collaborative scientific and commercial delivery effort to tackle a common single global challenge ie contraception, and to share learning for the benefit of the wider human community and the global ecosystem. This is very evident in the human contraception field where the developing world urgently requires the acquired benefits of more ideal, socio-economically and technologically appropriate contraception. The preventative focus must be strongly supported for human population control and probably by logical extension for better, sustainable animal welfare and population ecosystem animal management.

This human contraception driver is bringing new, global science to bear as exemplified by the present focus on germinal cell peptide technology at the University of Newcastle, NSW, Australia. It is recommended that MLA seek to explore collaboration with this research effort by Professor R J (John) Aitken et al, using their basic research knowledge to fund appropriate applications to production animals by other scientists in the large production animal field.

Collaboration will also help drive innovation and cross- fertilization of ideas. This has already led scientists to explore the new avenue of porcine zona pellucida (PZP) vaccine applied, to female cattle only, at a “proof of principle” level to search out advantages for the industry. Thus sterility as a goal may be achievable for targeted groups of females, eg cull heifers and cull/aged cows, given that this vaccine can be safely administered during a current pregnancy without abortion. Further support for this endeavour is recommended despite some identified commercial risk elements.

The issues of cost and ease of use of any contraceptive technology intervention(s) is an identified risk issue for red meat producers. Any intervention(s) to be researched must at least be screened from this likely cost perspective initially, monitored as to the degree of difficulty factor for commercialisation and its likely cost influence on any final product and assessed for its likely/projected adoption outcome/pathway. This report prepared a draft industry usage specification (based on northern cattle production and reported in the Methodology section) as an attempt to help think through and design specified outcomes/products/tools for and from research planning.

However the industry may well pursue (or be forced by public exposure to pursue) a direct self-help paradigm when seriously engaged with the welfare issues, by, for example, moving to the system of feedlot finishing young adult bulls for the export meat trade – see Project B.NBP.0486. This would drive both less castration, better productivity and a least cost win - win outcome where no additional technological innovation, incurring direct cost, was required for better male cattle welfare management. Despite these inherent attractions this strategy will not appeal to all producers, especially those apprehensive of managing mobs of entire males or of selling “bull beef”! Thus flexibility and a multi-pronged approach remain crucial.

Positive recommendations were delivered across the range of the scientific technology review areas with the aim to inform and help focus and direct, future animal welfare investments by MLA management over the 2015 - 2020 strategic planning period.

Current and potential future investments were considered together under specific science/technology subject areas and a broad portfolio approach was adopted by this review, unless the idea was historically clearly contraindicated eg intra-uterine devices (IUDs)

Recommendations cover applied/commercial delivery R&D, together with more strategic and basic research strategies. The need at this early (proof of principle/prototype) stage for a broad portfolio of investments, across most scientific approaches, is evident. This is particularly so as commercial outcomes to meet the preferred industry specification(s) have already in the past proven difficult to achieve eg Vaxstrate® circa 1990 requiring a minimum 2 vaccination shots.

An absolute first-up priority recommendation is given to GnRH chemical agonists which the review identified as an already proven in principle technology for production animals with a high likelihood of commercial success. Direct chemical reproductive intervention (with full potential for reversibility) will make this a preferred technology, if costs can be constrained.

The future focus with agonist technology should be to consider, cost, and, as appropriate/achievable commercially, test any/all other relevant high potency agonists eg Histrelin. This may help deliver an economically viable, lower input cost, sustained release product. Work should also be considered in rams to meet an identified need for later castration of surplus entire animals in seed stock flocks.

The industry best practice for implementation of a commercially efficacious and cost competitive product(s) should be researched using a replicated property demonstration sites approach. This would test proposed industry specifications for use against product claims for efficacy, duration of effect, reversibility etc in real industry situations and thereby promote innovative adoption.

The immediate goal in female cattle, applicable across the entire Australian herd, is a 100% reliable ie high efficacy, depot agonist contraceptive for heifers which facilitates achieving optimal joining weights without unwanted pregnancy and with preferred seasonal joining management ie timing. This requires well-defined contraception with reversibility around a predictable end point time span eg, 6, 12 or 15 months +/- 1month. That would provide herd owners/management with a strong and controlling opportunity to reliably align heifer breeding (joining then calving) with the feed resource base and optimise management to deliver sustained female lifetime reproductive performance.

GnRH immuno-contraceptive vaccines are currently marketed for short-term (up to 100+ days) reproductive behaviour management (both male and female) and female contraception, following a priming and second booster vaccination. This is commercial proof of principle AND practice!! A key determinant must be the technical capacity, in cattle in particular, to achieve high vaccine efficacy (eg animals remain non-pregnant) and to retain that capacity (ie animals remain non-pregnant over time, say 365 days) so as to achieve a definitive, specified industry outcome.

MLA may advisedly need to consider also, further performance enhancing technological innovations, such as those listed for discussion, to better achieve these outcomes. However, ultimately, reliance on an immunological response, in cattle in particular, for efficacious and highly reliable, 0.5 -2 years+ contraception may prove elusive. The “non-responder” outcome observed using adjuvanted GnRH vaccine may demand that alternative innovations eg DNA vaccines, be tried. Indeed ultimate judgements to curtail MLA/industry investment could be necessary. This will arise if commercialisation outcomes compatible with industry specification performance criteria cannot be achieved when based on reliance on poorly predictive, poorly quantifiable immunological responses.

However, current and necessary future commercial investments which trial applications in this field should be facilitated as far as is jointly possible, eg using MLA Donor Company support, to ensure maximum opportunity to reach these desirable longer term contraception outcomes. This would also signal industry support for such outcomes without which continuing commercial investment may falter.

GnRH toxin conjugates are a product technology of less than a decade in concept and development. Professor Terry Nett and colleagues at Colorado State University, USA, have now refined applications to the prototype product stage and have expressed renewed interest in commercial application studies in livestock. As this technology claims to functionally ablate the pituitary gonadotrophe cells in vivo (thus potentially inducing sterility) its putative value in terms of equivalence with male surgical castration is enormous. Re-engaging with this research group, as detailed in this report, is recommended based on a careful and critical appraisal of the developmental progress to date under commercial – in – confidence arrangements.

Kisspeptins and the hypothalamic kisspeptin receptor have emerged in basic science and medical publications only since 1996. This group of peptide hormones present new prospects for broad central (“upstream”) control of reproduction through disabling sterility outcomes, based on immune-contraception or chemical antagonists. A detailed scoping study followed by a strategic basic research approach, using sheep as the experimental/production animal model, is recommended. This would explore contraceptive potential beyond current and published studies in laboratory animals. It may help define and harness synergistic central control mechanisms in the hypothalamic/pituitary neurophysiologic drivers of puberty and reproduction.

All the above prioritised lines of research (GnRH agonists, GnRH vaccines, GnRH toxin conjugates and kisspeptins in that order) target sophisticated central nervous system neuro-hormonal control mechanisms, which have already proven to present various challenges to achieving effective, definitive control but which are attractive nonetheless for their generic potentialities.

However the contraceptive field is also wide open to and replete with other technologies targeting gonads or other peripheral reproductive organs. These are broadly considered from both female and male perspectives in the literature review process in the report.

The most widely explored ovarian target is the female egg zona pellucida glycoprotein complex. Multiple species have been targeted over 30+ years with considerable success but a PZP antigen vaccine for domestic cattle remains a developmental application challenge. Currently scientists are conducting proof of principle studies and when that is completed the greater challenge of industry application research linked with commercialisation will need to be addressed.

MLA levy funding and/or MLA Donor Company support for PZP vaccine is encouraged by this report, as is commercial co-operation. The potential application also to the southern beef industry for cull cows and/or heifers may assist in developing commercial interest and possible venture capital.

Porcine zona pellucida vaccine is therefore targeted for the cattle industry as the priority applied R&D field for short-term investment aimed at female peripheral reproduction targets.

In contrast more basic research should be contracted around the anti-implantation (uterine endometrium as target) approach and, more particularly, the broad gonadal germ cell contraceptive strategy developed in laboratory animals by Professor R.J(John) Aitken at Newcastle (NSW) University. An opportunity for future collaboration based on this science is outlined in the recommendations and should be pursued in a timely way in 2014 with CSIRO, whose Food Futures Flagship Program commissioned the University of Newcastle basic science effort in this field over the last 3-4 years. Large animal application studies, particularly in male cattle, are envisaged as the next developmental stage of the work.

The focus on male cattle is warranted as, apart from the GnRH vaccine approach which is the single most likely to succeed in terms of being the most commercially advanced technology, there are no other advanced male non-hormonal contraceptive technologies which commercially could be simply adapted for livestock. This also reflects the paucity of human male contraceptive R&D in general over the years, which is only now being redressed eg Li-Hua Yang et al (2011).

This recommendation for investment in a new technology aimed specifically at the gonads (both male and female) is consistent with a broad portfolio approach now encouraged. This will help to increase the likelihood of successful industry applications emerging from the science/R&D investment pipeline at a later date. Such multi-pronged strategies will need industry support to facilitate and to justify the investment quantum required, over the effective period of the current five year plan and probably beyond.

Therefore given likely extended timelines for all new R&D it is strongly recommended that the proposals on development of channel webbing for female cattle and the marketing of entire males by 2-2.5 years of age covered in this report be supported by industry leaders and MLA. They should be rapidly advanced for industry implementation as an immediate response to reduce the necessity for and/or frequency of spaying and castration. This would help to negate likely animal welfare criticism of industry, particularly in northern Australia, for failing to act on castration and spaying of older animals, whilst awaiting research on full replacement technologies.

This review has a final dilemma of multiple competing technological choices, many of which have not even been adequately investigated in commercial livestock. This presents an ongoing, challenging and complex decision making paradigm which will need close on-going R&D managerial oversight by MLA. Attention must first be paid to specified delivery outcomes at industry herd/flock level and then to the likely product/service outcome, it's scientific and commercial viability, including efficacy and likely cost/benefits for both suppliers and users. A comprehensive win-win scenario alone will suffice!!!

The R&D investment and product development decision making frameworks will inevitably tend towards "picking winners" but with limited dollars (from all stakeholders) "c'est la vie"!! However, the preferred R&D pipeline process of defining biological limitations clearly and quickly and then being willing to "drop off" the demonstrable (on the evidence) unlikely "stayer" contenders early, are necessary disciplines to achieving successful commercial and industry outcomes.

MLA (on behalf of industry) has a vital and pivotal role to broker and facilitate the factual, critical science investment and the related, necessary, frank discussions with commerce and industry to define and follow key critical developmental pathway(s) to success in the longer term.

Welfare (including castration and spaying) replacement technologies MUST emerge to facilitate better industry productivity, including labour productivity, NOT to primarily placate an animal welfare or other activist position. Better animal welfare and associated health outcomes, with concurrent improved productivity – both animal and animal management - will then recommend and sell themselves to MLA members who want to run competitive livestock businesses.

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Introduction

Surgical husbandry procedures definitely impact animal welfare. The older animal is more impacted consequent on the mass of tissue incised or excised. The animal welfare sequelae, include acute and post-acute pain, blood loss and particularly post-surgical complications, associated with open or even ligating surgical husbandry procedures eg rings and tension bands. The impact may extend to poorer flock and herd productivity and, through losses in particular eg post-marking tetanus outbreaks, to reduced financial performance. Further, the worst welfare outcomes present a potential risk to the red meat industry's community perception and status.

Any proactive industry investment to maintain or improve on-farm animal welfare is therefore a positive step to assist industry in remaining sustainable, more productive and in addressing public interest in acceptable on-farm practices.

Research and development (R&D) investment in particular can and does deliver outcomes which over time meet these economic, technical and social expectations.

However any grass-roots changes to on-farm husbandry procedures to improve welfare MUST be practical and economical, with long term advantages for producers.

A useful case study and example is the characterisation of the polled condition in cattle, sheep and goats, the scientific definition of the broad advantages on and off-farm for "polled-ness" and more recently in cattle the genomic science that can define and allow specific selection for the polled condition in Brahman and other breeds of cattle. Thus a "no-dehorning" status for an outcome focused 21st Century cattle/livestock industry is conceivable!!

The surgical castration of larger males (>6 months) and the ovariectomy of female cattle in far northern Australia are still considered vitally necessary for manageable and profitable extensive production systems. However even the current improved procedures do cause significant though transient pain, blood and productivity loss and in some cases wound infection, spreading of blood borne diseases and death.

MLA has in the last decade and a half invested funds in R&D to improve surgical husbandry practice eg Willis dropped ovary technique (WDOT) and to attempt to find non-surgical alternatives including bovine contraception.

However, the backdrop scientific fields are large and diverse, driven globally by significant medical research eg for human population control, and such things as conservation, public health (rabies control) and the environment eg wildlife population control, domestic strays, urban and peri-urban animal population control etc. Many, many options have been explored in animals and man over the last 50 years but none have been pursued in animals to fully emerge commercially as a successful replacement solution applicable to commercial livestock at the farm level.

Alternative, genuinely commercial, non-surgical solutions ie replacements, are the ultimate outcomes envisaged by MLA in the Animal Welfare Strategic Plan 2015-2020 from this total endeavour.

Background

The internal review of MLA funded projects, documented below as part of this project brief, is delivered chronologically. It indicates a history of industry led research interest in control of female reproduction through oestrous suppression from the mid to late 1990s into the early 21st Century.

This resulted in the validation and subsequent widespread adoption of the Willis Dropped Ovary Technique (WDOT) as a surgical improvement over a cruder history of use of flank spaying. No other long-term sustainable industry R&D outcomes emerged from that time, though Vaxstrate® GnRH vaccine was developed and marketed as a world first here in Australia.

In the period 2005-2011 a number of influences were abroad, which heightened the livestock industry focus on the welfare of farmed livestock. These included:

The PETA campaign against the wool industry with respect to breech mulesing and evidence of the powerful influence of media, markets and special interest advocacy groups,

The strain on industry, researchers and Australian Wool Innovation (AWI) to fast-track research driven solutions to replace mulesing with one or more acceptable new alternatives,

The Commonwealth Government developed the first Australian Animal Welfare Strategy (AAWS) as a national, comprehensive, multi-faceted approach to animal welfare policy, strategy, research and communication in collaboration with State jurisdictions and engaging many industry and advocacy groups,

The advent under AAWS of the Animal Welfare Standards and Guidelines consultative process whereby the former voluntary Codes of Practice for Animal Welfare, historically produced and published by the Commonwealth Department of Primary Industries (now DAFF), were to be replaced with new defined and enforceable criteria (Standards) and Guidelines for animal welfare,

Transfer of the Standards and Guidelines management process to Animal Health Australia Pty Ltd from DAFF and thereby more forcefully engaging industry in the consultative process. This involved confronting issues such as compliance, uniform State legislation, best practice, out-dated or unacceptable practice, the role of science, pain relief, who represents whom (Councils vs State organisations), etc,etc.

The ban live-exports campaign by the World Society for the Protection of Animals (WSPA) in 2009 and 2010 with the subsequent live cattle to Indonesia crisis generated by Animals Australia from cruelty video taken in Indonesian slaughter houses and programmed for public exposure by the ABC Four Corners program in mid-2011.

These influences and the heightened industry awareness generally of animal welfare were brought together and addressed by SCA/CCA and MLA in the Sandra Wellsman (Frontiers Insight P/L) review projects (B.AWW.0204, 2010 and B.AWW.0228, 2013) which sought to crystallise the welfare future and guide MLA and industry policy thinking, towards a more targeted, thoughtful and philosophically unified approach and consequent public position.

Animal welfare activism was recognised by all concerned as a 21st Century reality. Around it industry must fashion medium to long term policies and practices (including R&D investments), whilst seeking to provide improved reputational and market outcomes and public perceptions in the new era of social media, talk-back radio, adversarial public media, political lobbying and animal rights legal advocacy.

In the research area industry should seek to proactively invest in 3 R's strategies ie replace, reduce, refine by which a much smaller or nil target for future public criticism could be presented eg all cattle polled. Research which would NOT change the perception of the man or woman in the street, eg defining animal thinking (mentation), should be avoided - as a likely poor investment!!

MLA commenced a number of new investments with a replacement focus in the period 2006-2011 under the leadership of Dr Ian Johnsson LPI General Manager. These included the genomics of the polled condition in Brahman cattle, GnRH-Pokeweed Antiviral Protein basic research and Gonacon™ potential application to female cattle contraception.

This Wellsman animal welfare review process was also embedded in the thinking of the 2012-2016 MLA Animal Welfare Strategic Plan approved by industry peak councils and the MLA Board of Management.

These multiple elements and influences have led then to this current project - to review relevant MLA Welfare R&D investments and to recommend revised strategies for investment over the next five years. The focus is particularly directed at seeking research driven alternatives to the current industry practices of spaying of females and castration of male animals, particularly cattle over the age of 6 months or older at first muster. These are and were judged to be on-going areas of relative animal welfare risk for industry, particularly given the negotiations around the development of the Cattle and Sheep Standards and Guidelines which have been refined after the public consultation stage.

Appropriately specified and cost beneficial research with a focus towards on-farm outcomes will be sought for ultimate ease of industry adoption. However a major challenge is likely to remain ie that of industry acceptance and implementation. The most compelling outcome(s) will surely be productivity driven with a welfare (replacement) bonus rather than welfare driven alone. However, welfare awareness by industry is growing, albeit reluctantly and of necessity, and the level of that awareness will be influential in future adoption decision making on farm.

Project Objectives

The project title encapsulates the thrust of the project, that is, to review the alternatives to castration and spaying of ruminants.

Specifically the contract objectives were delineated as follows;

- to review the scientific literature,
- to review and critique past and current research funded by MLA in this area,
- to assess the likelihood of success due to technical issues for current research,
- to identify risks of failure due to technical issues for current research,
- to examine potential solutions for down-side risk of future change of community views,
- to identify promising areas, if any, for future short and long term R&D.

Further the project should interact with leading research scientists in Australia to solicit and document their views about gaps and/or weaknesses in the current program of R&D and opinion(s) on R&D that MLA should or could support.

Finally the project should identify any promising areas for short and/or long term R&D investments to contribute to a future for industry which includes potentially viable “non-surgical” replacement alternatives.

Methodology

Given the project's review objectives a mixture of data and opinion collection methods were applied including the following;

An initial scene setting science focus group meeting was convened on 4th March 2013 from 1.30 - 5.30pm by Dr J T Rothwell at the University of Queensland to introduce and canvas the project objectives by scoping the field of reproduction control. This meeting with 8 attendees initiated the project. Key topic areas were identified courtesy of the invited scientists.

Using iSearch, then the MLA Website R&D search engine and cross-checking the corporate knowledge of MLA staff covering the last 15 years, a funded projects internal review was achieved.

A de novo literature revue was conducted by a search of all available published refereed journal literature through "Summon" at the University of Queensland library.

The overall literature search brief for this de novo revue was constructed as follows:

General search terms were chosen and tested to finally include the following key words – contraception (man, animals, later sub-search cattle/sheep), pregnancy prevention (as a co-term for contraception), immuno-contraception (as an independent primary search term + man ,animals then sub-search cattle/sheep),review (to access additional papers other than specific primary publications), then sterility/male/female (man, animals + sub-search cattle/sheep).

The following primary search terms were avoided to constrain and focus the searches, after initial screening indicated large irrelevancies eg human female steroid hormone contraception. These excluded search terms included chemical, fertility, fertility control, non-surgical, contraceptive, non-steroidal, non-barrier, intercourse independent, reversible and ruminants.

Technically discrete terms were only used for sub-search purposes where indicated when large lists were generated from specific primary searches or where specific species applications were sought, say GnRH agonists in cattle. Examples include GnRH, GnRH agonists, deslorelin, zona pellucida, oocyte, sperm, etc

An iterative approach was followed until key review references were located (often from more than one search process) and these were read and used to inform any more detailed searches or to locate individual relevant publications.

No search process can be considered exhaustive and this one was only conducted in English, using one University e-library capacity and without the benefit of critical and complementary peer review prior to submission of the report to MLA.

However Dr Peter Chenoweth and Dr Scott Norman from CSU Wagga Wagga kindly provided for reference a near final draft pre-publication copy of a draft book Chapter entitled "Male Animal Contraception" from their new book on animal andrology. This allowed the reviewer to independently compare process outcomes in one key half- area and to reasonably establish the arrival of the literature review at an acceptably comprehensive result.

After the three studies above were completed and the critical reviews summarised, key authors and/or deemed relevant academics at each Australian veterinary school were requested by e-mail to address the project brief and provide comment/feed-back or add additional review resources for the project.

Correspondence by email was initiated with Australian and cited overseas published scientists in an endeavour to tease out new work as yet unpublished, to seek information on any patent or particular commercialisation activities and to enquire of possible or actual studies with, or with application to, ruminant species.

As possible and within budget constraints interactions/interviews were attempted/conducted with the following relevant active Australian scientists;

Professor Michael K. Holland, University of Queensland, Brisbane, Qld.
Professor Michael D'Occhio, UQ Brisbane Qld
Professor Michael McGowan UQ, Brisbane, Qld.
Dr R. J. (John) Aitkin, University of Newcastle, NSW.
Dr Eileen A. McLaughlin, University of Newcastle, NSW.
Dr Scott T. Norman, Charles Sturt University, Wagga Wagga, NSW.
Professor Toth, University of Queensland (contact of MKH).
Dr Peter Chenoweth CSU Wagga Wagga, NSW.
Dr Geoffry Fordyce UQ, Charters Towers Qld.
Dr Teresa Collins, Murdoch University Perth WA.

In addition veterinarians actively consulting to the Northern Australian cattle industry were questioned about the practical adoption issues for industry of potential contraceptive technologies. Dr Alastair Henderson, Dr Geoff Niethe and Dr Peter Letchford specifically were consulted at the 2013 Australian Cattle Veterinarians Conference in Darwin, June, 2013. This "Focus on Fertility" reproduction themed conference included one paper entitled "Oestrus suppression in females – Bopriva and beyond" dealing with control of female reproduction presented by Dr Neil Charman from Zoetis P/Ltd.

Part of the methodological approach during the project was to crystallize the preferred outcome for industry from the R&D, in discussion with scientists and professionals closely engaged with current industry practices. This future tool/product specification process is defined below (as at 3/7/2013) and should and will be used to help clarify all project evaluations and new project recommendations.

The detail for the PREFERRED OUTCOME FOR INDUSTRY from R&D is as follows:

A full 12 months minimum contraceptive (non-pregnant) effect, preferably 100% effective, ie low (≤ 2%) to nil failure rate (pregnant) or very few non-responders

Applicable to both heifers and cows ie effective across weight range 180Kg – 600+Kg

Not technically mandated for application to only young animals less than 6 months of age

Not affected by big variations in nutrition/body condition score eg lower or variable immune response

A defined permanency of effect pattern for confident application by industry eg 1 year ± 1month or 2 years ± 6 months or life-time effect (sterility)

Preferably the same product applicable to both females and males eg GnRH agonist has differing response in males compared with GnRH vaccines

Reversible for females, particularly breeders both heifers and cows, with a predictable and neat cut-off or cessation of effect pattern eg within one oestrus cycle

A non-reversible technology is permissible for cull males (steers and wethers), cull heifers and cull or aged cows ie sterility including steroid hormone ablation rather than reversible infertility

Low labour input ie one "shot" operation or one yarding per year - lesser option of two "shots" if they can be up to 12+ months apart.

Low degree of difficulty for application ie prefer ear implant vs needleless injection (mucosal, percutaneous, intra-vaginal, etc,) vs injection subcutaneous, intramuscular or intra-peritoneal

Safe for animal use

Safe for operator use or can be made safe ie no OH&S issues

Cost per se has NOT been included in this technical specification and is ultimately a balance of input cost(s) vs benefit(s) determined by product/tool design and performance criteria at the individual animal and whole herd/farm levels. Potential management driven productivity and marketing improvements will figure prominently in assessing benefits as will the intangibles of better welfare outcomes.

Results

Reproduction Control - Science Focus Group Meeting

This meeting was conducted as the official start of the project on Monday 4th March 2013. It was held at the University of Queensland, St Lucia and was convened by MLA (Dr J T Rothwell) with a verbal confidentiality agreement in place given by those present.

The meeting engaged CSIRO/UQ researchers currently funded by MLA (Michael D'Occhio) or working independently in the field of contraception (Michael Holland, Michael McGowan, Rachel Pegha –a post-doc for Professor Toth) plus consultants to MLA (Walker and Banney).

The multi-factorial nature of, and possibilities for, a viable industry application(s), ie a transformative replacement alternative to castration and/or spaying, was described/discussed at length.

Factors identified included available and applicable contraceptive science technologies and approaches, acceptability to producers and consumers, added costs across and within the supply chain, variability and duration of biological effect(s), including in both males and females possible permanent sterility, differing bovine immune and gender specific responses, commercialisation opportunities and risks, on-farm management reward for success and "degree of difficulty", OH&S and food safety issues etc.

Table 1 Summary of the meeting's considerations on any known technology category and its gender suitability with comments on the meeting participants views of success/risk elements for each category.

Category	Suitability	Comments
GnRH agonist	Females	Compounds such as Deslorelin work but are costly. Not regarded as a 'nasty'. Has good potential.
GnRH vaccine	Males & females	Gonacon has the potential to work in both genders. New work with bull calves now complete. It does produce a Johnes disease titre with implications for live export market. Has good potential.
Pokeweed Antiviral Protein (PAP)	Males & females	Ablation technique using a cytotoxic compound which may not be acceptable to community. Some doubt on the validity of recent US data.
Ovarian necrosis	Females	Injection into testes works so trialling in ovaries.
Zona pellucida	Females	Looks to cause the permanent death of follicle function.
LH receptor vaccine	Males & females	
LH vaccine	Males & females	

MLA Current and Retrospective Projects Review

Historic MLA funded projects were reviewed early in this investigation to revise and review the research effort to date (2013) via publications etc and to delineate areas of specific interest for the concurrent published scientific literature review, using a largely common key word search criterion.

A comprehensive coverage was determined by use of the MLA document search tool iSearch, together with a similar screening process using the MLA Website which utilised the R&D publications search function. Both these searches used the key words itemised as follows:

Pregnancy prevention
Contraception
Oestrous suppression
Immuno-contraception
Sterility/Fertility
Non-surgical castration/spaying
Spaying
Castration
Animals/Cattle
Chemical/Agonists/Antagonists
Note: The search terms man/human, male, female and review publication, were restricted for use as qualifying terms in the wider scientific literature search only.

Finally email correspondence or personal enquiry of research scientists or MLA staff, were together used to “double-check” the bona fides of the search and to ensure a comprehensive and peer reviewed outcome.

Retrospective Projects

The project portfolio resulting from this search process in order ancient to modern is as follows;

Project NAP3.105 circa 1996-98 Oestrous suppression and pregnancy prevention in cattle. Value $100K See final report including publications.
This project examined oestrous suppression and pregnancy prevention in northern Australian cattle especially in the light of the increasing need for export heifers to meet the market specification of “non-pregnant”. Specifically it tested three (3) alternate technologies for pregnancy prevention in turn-off heifers and cull cows, those being WDOT (Willis Spay)), an intrauterine device (IUD) and a GnRH agonist bio-implant compared with control groups consisting of para-lumbar (flank, heifers) and vaginal (passage, cows) spaying. 4% bulls were used for 12 months of joining. There were 3 different property replications.

It was primarily concluded “from this project that the WDOT or Willis Spay procedure is the preferred surgical method for contraception in heifers and cows and should be actively promoted.”

It should be noted that this evolution has since largely transpired in industry supported by veterinary and lay provider skill development in, and enthusiasm for, the WDOT in preference to other surgical interventions. However post-procedure negative sequelae including haemorrhage, mortality and pregnancy do still occur as evidenced in published comparative welfare focused field studies of WDOT (2003-2006) led by Professor M. McGowan from University of Queensland.

Second the IUD was inefficient and difficult to apply as a contraceptive technology under northern Australian conditions. Substantial pregnancy rates occurred in up to 40% of heifers and cows in lesser groups, ie those animals able to be successfully implanted. It was NOT recommended for further industry use in Brahman derived breeders. IUDs are not available to or used by industry since this work.

Finally a GnRH agonist bio-implant successfully suppressed ovarian follicular growth and induced infertility in the majority of heifers and cows. Pregnancy rates for heifers and cows at the end of the

study ranged from 10% (2 properties) to 25% (1 property). The latter result was considered to be due to differences in implant formulation. Interestingly first pregnancies occurred after some 240days (2 properties) and 340 days (1 property with higher dose rate 12mg implant)

It was concluded that “the GnRH agonist bio-implant has considerable potential as the next generation, non-surgical technology for oestrous suppression and pregnancy prevention in cattle. An outstanding advantage of fertility control with the GnRH agonist is the reversibility, which will provide new options for reproductive management of both breeder and non-breeder heifers and cows in extensive beef herds.”

Unfortunately, no consideration of bio-implant cost or commercial availability, nor a technology development pathway is provided in the project report.

This supply side risk was certainly operative in the period 1999-2009 as evident a decade later when MLA staff (Ian Johnsson, Johann Schroder and Keith Walker) sought new information on lack of product availability to industry to that point in time. High product cost was cited as the primary determinant of unavailability together with technical issues of bio-implant dose release dynamics vis a vis whole product cost and commercial profitability. These commercial issues for cattle applications had not been explored by Peptech P/L Sydney in contrast to successful product development for dogs.

Project Related Publications

D’Occhio MJ, Aspden WJ and Whyte TR 1996 Controlled, reversible suppression of estrous cycles in beef heifers and cows using agonists of gonadotropin-releasing hormone. J. of Animal Science 74 (1): 218-225
D’Occhio MJ, Fordyce G, Whyte TR, Aspden WJ, and Trigg TE 2000 Reproductive responses of cattle to GnRH agonists. Animal Reproduction Science 60-61:433-442.
D’Occhio MJ, Fordyce G, Whyte TR, Jubb TF, Fitzpatrick LA and Cooper NJ et al 2002 Use of GnRH agonist implants for long-term suppression of fertility in extensively managed heifers and cows. Animal Reproduction Science 74:151-162

Project NAP 3.110 circa January 1998 to December 1998 Evaluation of a GnRH agonist bio-implant for oestrous suppression and pregnancy prevention in heifers. Value $30K See Final Report including publications above.

This project was complementary to Project NAP3.105 and sought to establish a dose response relationship for the agonist (deslorelin) in the bio-implant and to determine an upper dose which could induce an effective contraceptive effect in heifers for 12 months or longer. Additionally any negating effect of good nutrition was examined in the last 3 months of treatment with live-weight gain of 0.63+or-0.02kg/day. 3, 6 and12mg bio-implant doses were chosen.

Control heifers conceived throughout the trail with no biological or environmental constraints. The GnRH agonist low dose treatment suppressed conception for approximately 3 months while the medium dose treatment prevented pregnancy for around 6 months. Pregnancies at 12 months were Low dose, 36/48 (75%): Medium dose, 26/48 (54%) and High dose, 3/45 (6%). Thus at 12 months in excess of 90% of heifers in the GnRH agonist High dose treatment continued to have suppressed ovarian activity and there were no pregnancies detected at the previous 8 month post-treatment observation ie the three pregnancies occurred in the last third of the year-long observation and continuous mating period.

Good nutrition in the last 3 months of observation was imputed to have NOT affected contraceptive efficacy but the body weight gains were modest though rising and failure of contraception (all groups) plus slightly greater numbers of pregnancies in controls were concentrated in that specific period. However deslorelin bio-implant release may also have been in decline in that period thus directly influencing conception rates. Thus the nutrition effect per se MAY require a better designed experimental approach for clarity. However significant contraception did continue to be exerted and was dose related.

Cost/benefit information was noted as NOT being available at the time. Thus proof of action in principle had been scientifically achieved with the two projects but there was no forward plan for delivering an industry outcome via a commercial deliverable.

This project conclusion was reached shortly prior to the advent of the MLA Donor Co. thereby leaving staff no supportive commercial negotiation structure to facilitate discussions around a commercial development plan.

Project Related Publication
D'Occhio MJ (1999) GnRH agonist bio-implants for control of reproduction in heifers and cows.The North Australia Program, 1998 Review of Reproduction and genetics (Editor S. Blakely), NAP Occasional Publication No 8, 87-91.

Project NAP3.120 circa 2003-2004 Immunocastration of male cattle.Value $46K See Final Report including 25 reference bibliography.
This project looked at the GnRH vaccine Improvac™ (CSL P/L, Victoria and registered for use in male pigs) as applied to 24 month old Bos Taurus x Africander bulls and compared growth, body and carcase features in two small groups (n=7 or 8 Controls vs GnRH immunocastrates). Ultrasonographic and pre- and post-slaughter physical measurements eg EMA, P8 fat & 13th rib fat, were collected including testicular parameters and weight.

The project showed that immunocastration of sexually mature bulls results in a progressive shift in body and carcase characteristics from those of entire bulls to those of steers. Ultrasonography was successfully used to track the changes in body composition and would allow decision making on expected carcase measures and fitness of live individuals for particular value- based market grid specifications eg for immune-castrated bulls being finished in feedlots.

Significant limitations were found in the vaccine technology as adapted to cattle. Poor GnRH anti-body responses with both Improvac and a prototype vaccine for cattle dictated a multi-dose vaccination regime (Improvac n=5 doses) or a poor response (prototype vaccine n=2 doses).

Recommendation was made for a review of immuno-castration technology and possible further industry R&D investment.

The project was a useful start-up field application study in cattle but appears to have been premature in terms of the maturity of immuno-contraceptive technology development in Australia.

The prototype developer CSL P/L was subsequently acquired by Pfizer Animal Health soon after this time and the reviewer is aware that the prototype CSL product was developed to market (2010 New Zealand, 2011 Australia) as Bopriva® by Pfizer Animal Health (now Zoetis P/L 2012). This demonstrates significant evidence of commercial drivers for success in this field locally in Australia/New Zealand. This capacity is crucial for any tangible product delivery to the cattle industry

Project related Publication
D'Occhio MJ, Aspden WJ et al 2001 Sustained testicular atrophy in bulls actively immunized against GnRH: Potential to control carcase charactistics. Animal Reproduction Science 66:47 -58

Project B.AHW.0155 2006 to 2012. Value $40K Title GnRH-Pokeweed Anti-viral Protein(PAP) Treatment for non-surgical Castration of Sheep and Cattle. Dr Michael D'Occhio (Uni.Qld) and Prof.Terry Nett, Colorado State University as collaborator.
The aim of this project was to use the plant derived PAP (which is a potent ribosome-inactivating protein and through blocking ribosomal protein synthesis is directly cytotoxic) when conjugated to a GnRH agonist viz deslorelin to target GnRH receptors on cells in the pituitary and thus ablate (kill) the gonadotroph secreting cells thereby inducing lack of or complete suppression of GnRH. This would diminish or suppress gonad secretion of LH and FSH and thereby induce infertility/sterility by pituitary hypogonadism.

The collaboration employed, and MLA supported, a UQ doctoral student in Prof. Nett's Animal Reproduction and Biotechnology Laboratory in Colorado State University to pursue the concept following earlier conceptual studies in prostate cell lines, GnRH and GnRH agonist sensitive tumours in humans eg breast, ovary, endometrium and prostate and reproductive function in adult male dogs. (See Review Harrison GS et al GnRH and its receptor in normal and malignant cells. Endocrine Related Cancer (2004) 11:725-748)

A number of developmental hurdles were encountered eg the need to derive and utilise recombinant rather than native plant derived PAP, consideration of the alternative use of Diptheria toxin, use of the agonist or only the GnRH-rPAP conjugate etc. The doctoral student graduated successfully using the developmental science conducted in the USA and mouse studies were reported informally to provide evidence of scientific proof of principle. The project was unable to deliver a definitive timetable for agreed large animal application studies. More recent correspondence with Prof. Terry Nett indicates that a biotech venture Company now exists and their CEO has personally expressed interested to collaborate with Australia in the necessary future testing in production animals. The matter is dealt with in the recommendations section.

Current MLA R&D Levy and MDC (MLA Donor Company) Funded Projects

B.NBP.0486 circa 2010 -2013 Value $948.5K "Growth and meat quality of grain finished entire male Bos indicus cattle." Author Prof. Lee A. Fitzpatrick, James Cook University Townsville.

This project addressed several major colliding concerns for northern producers – available market outlets for cattle, castration as an animal welfare issue, productivity gain from improved growth rates in entire males and consequent energetic efficiency with less greenhouse gas emissions. The hypothesis tested was that young entire *Bos indicus* males can deliver increased returns with minimal loss of meat quality, while simultaneously meeting and by avoidance exceeding new welfare standards for castration at > 12 months of age or at first muster ie requirement for effective pain relief.

Generally entire bulls generated 11% higher average daily gain and some $52 higher gross value than comparable steers despite some poorer MSA grading scores for entire bulls, particularly for "subcutaneous fat depth out of specification", viz 31% of entire bulls ungraded for MSA versus 14% of castrate steers.

The free unvalued benefit on farm of avoiding the need to castrate older weaners (approx. 200Kg at 8-12 months in this study) by this "replacement" strategy, strongly suggests that the Australian industry should "rethink" the possibilities of this approach. It is common elsewhere in the world and did not create any significant behavioural differences between the entire and castrate treatment groups in this study.

As the northern industry has significant numbers of missed muster and marking, entire males (so called mickey bulls) each year (estimated at least 8-10,000 slaughtered annually) why not value add at least this population of animals by a deliberate grain finishing management strategy with local slaughter???

Whilst mustering efficiency is a key proviso, the widespread gradual acceptance of such a marketing strategy would allow producers to choose to forego castration and move increasing numbers of males to the entire male marketing pathway. This in turn would directly "replace" castration as a completely non-essential, or at least partly optional, management procedure and give industry a highly desirable least cost replacement strategy.

This win – win strategy has been sketched out in detail by this project but the issue of industry adoption and structures to achieve this outcome eg MSA criteria for *Bos indicus* bulls, remain to be

tackled. Initial vocal opposition to the project concept perhaps indicates some resistance to change which will need to be addressed. However the animal welfare imperative is clearly increasing nationally and has already demanded elsewhere in the world that cattle are not surgically castrated beyond a few weeks to months of age!! - see recommendations section.

B.AWW.0194 circa 2011-2013 Draft final report dated April 2013 Value $67.95K
Project Title Gonicon™ trial in heifers.
This project investigated the utility of this imported GnRH immune-contraceptive vaccine to suppress ovarian activity over time (multiple months not weeks).The GnRH anti-body responses were measured progressively as was ovarian follicular activity.

The response to a single (3mg) vaccination was uniformly poor with only 1/10 heifers having a low ovarian follicle score. However, two vaccinations 60 days apart (2mg then 1mg dose regime) induced both significant anti-GnRH antibody titres in 6/9 heifers after second vaccination and ovarian follicular activity scores were suppressed for up to 330 days in 5/9 heifers.

The recommendation was made that a higher dose regime be examined in future, potentially 5mg and 3mg for primary and secondary vaccinations, as this was the first time that the product had been tested in cattle after being developed in the USA for white tailed deer and previously only tested in American bison (one vaccination of 1.8mg) as the nearest bovine relative.

The particular technical concerns arising are the failure to generate a “one-shot” response in a product designed for that outcome (an obvious on-farm marketing competitive advantage) and the concurrent generation of Johnes disease titres in response to the Mycobacterium avium included in the product adjuvant. This component has been shown to be causal in the “one shot” long term response in deer (Perry KR et al 2008 Proc.23RD Vertebr. Pest Conf.:253-256 (published at Univ. of Calif., Davis.) which had prior environmental exposure. These technical issues are canvassed in the final report discussion prior to the recommendation to repeat the work at higher doses (see above).

The more general concern, beyond proof of principle in cattle, is the likely commercial availability to industry in Australia. This particular product has no current linked commercial champion/owner as the inventor and patent holder is the USDA APHIS National Wildlife Research Center, Washington, USA which is not a commercial entity. However direct manufacture in Australia may be feasible and the Australian Invasive Animals CRC is currently actioning a research co-operation agreement with the USDA APHIS which may include product import/local manufacture options.

Further, registered use of Gonicon for cattle in Australia potentially conflicts (through M.avium adjuvant sero-reactivity) with Johnes Disease diagnostic capacity in any such vaccinated animals and this may therefore impede registration processes. However alternative adjuvants to prevent the M.avium exposure, serological or other tests to distinguish concurrent GnRH vaccinates from true Johnes Disease infected animals or other strategies eg repeat vaccination with anti-GnRH anti-body titre measurement to confirm vaccination status plus direct PCR on faeces to negate infected status, could all be considered. This could satisfy the low probability risks in the northern Australian herd where Gonicon is indicated for use, that contraceptive vaccination status and Johnes Disease status may clash.

Finally MLA have recently funded an extension of this project to test both higher doses and the entire male bovine response to this product. This work will be comparable with that of Price et al 2003, who looked at effects on aggressive behaviour in young bulls (vaccinated and unvaccinated) compared with steers with the vaccine being a progenitor similar to Gonicon™. Based on comparing anti-GnRH titre measurements this testing should provide some clarity around the nature and quantum of the Gonicon/bovine response and provide some additional technical insight. However it will not address the more general externalities touched on briefly above.

Project P.AWW.0219 circa 2013 Value $65.495K Project Title: Chemical Sterilisation as an alternative to spaying of heifers –

The aim of this project is to evaluate the effectiveness of individual animal trans-vaginal intra-ovarian injection of either zinc gluconate or calcium chloride to attempt to sterilise female cattle. This is a surgical spay alternative approach seen, and argued, as likely more welfare friendly by the proponents. However the technique has not been demonstrated in female cattle (or other female animals) before nor welfare measurements taken. Currently in the project proposal there is no positive WDOT control group only a sham injection negative control group proposed.

The approach is based on IVF techniques for harvesting oocytes by ultrasound guided ovarian needle puncture. It is technically feasible based only on comparable chemical testicular injection studies in male animals (see application for useful up to date bibliography mainly dated 2002 - 2011, which evidence preferred use in dogs for the chemicals proposed for use in this study.) This approach is technically difficult necessitating a veterinary approach, possibly marginal welfare gains compared with spaying per se and the likely cost based on the assumptions made about veterinary service provision availability. Even were it to prove highly successful, more rapid to perform, clearly more welfare friendly and be readily adopted by veterinarians eg to entirely replace WDOT surgical spaying as their preferred technique, it may NOT of necessity be widely adopted by the extensive northern cattle industry. This is due to the likely relatively high cost of time critical professional intervention(s) on-farm.

In addition it is ethically arguable (whatever the welfare measures outcome) whether the veterinary profession should be perceived to advocate a yet more complicated/costly and essentially tissue necrotising physical surgical intervention of the female of the species under the banner of better net animal welfare. This position may not be sympathetically received by beef producers, their wives in particular or the general public, if only on perception or anthropomorphic grounds. Therefore, at best this is as a possible refinement strategy which veterinarians may embrace and perhaps be able to sell to industry, rather than a true “common sense” replacement strategy driven by industry itself on the ground. At worst the bovine ovary may prove anatomically unsuitable for a central single necrotising injection done remotely by manipulation.

Notwithstanding this reviewer’s personal ambivalence, the project may well help some veterinarians provide a revised service provision package which would potentially reduce surgical WDOT spaying and the mortality from post-procedure haemorrhage etc associated with it, even for experienced operators with practised skill competency. However the development of and promotion to and training of under and post-graduates in per vagina channel webbing for cows and heifers rather than WDOT might be a more valuable academic contribution. This possibility should be further explored and was actively discussed with Dr Peter Letchford from Kununurra, WA at the 2013 ACV Darwin Conference (see recommendations).

MLA Donor Company Projects.
These projects are strictly commercial – in – confidence contracts and will therefore not be reviewed openly here. However the reviewer has whilst contracted by MLA been privy confidentially to the project applications and therefore able to assess the broad intent of the science. Thus it is possible to report that the two relevant projects are both projects with significant investments in published, different but well proven contraceptive technology with the common aim of significant product development and refinement and with a resulting marketable industry deliverable.

Industry will be engaged in field application studies during the APVMA product registration process and will be well positioned to consider adoption of both technologies within the life of the current MLA strategic plan (2012-2016), if and when final registration is achieved which is a timing proviso. On property usage in northern Australia could involve serial but overlapping concurrent use of both technologies for optimum management outcomes in my current view, depending on allowed label claims and final product costs. This situation bodes well for sensible commercial delivery to meet

expressed industry need at an important timing cross-road between the new Cattle Welfare Standards and Guidelines and the commercial availability of practical on–farm tools.

Conclusions from the MLA Current and Retrospective Projects Review

There were 3 past projects (1996 -2004) in this area which reflect where Australian science has led the field in animal applications eg agonists and immuno-contraception. However these failed to deliver sustainable red meat industry outcomes due to lack of follow-up commercialisation.

There was one cancelled project this science based review will recommend a re-consideration, particularly in the light of new commercial venture support.

There are 5 current new projects in total exploring various proven historic (n=3) and new (n=2) alternative technologies and which offer industry some renewed prospect of tangible commercial outcomes to use on-farm. Two in particular represent well developed, now commercially supported and potentially maturing possibilities for industry outcomes.

MLA should also consider other new additional investments in strategic science to increase, in the prospective scientific “pipeline”, options for industry in the science of “down-stream” gonadal (both testis and ovary) germinal cell or oocyte/sperm targets. (See the literature review and recommendations sections of this review report.) There are no past or current investments by MLA in these areas but other Australian scientists (both CSIRO and University academics) have been funded in these areas of more basic and strategic reproduction science, including control/contraception.

The industry needs and perspective on adoption drivers may well vary in future but a spread of 4 new (in terms of mode of action/science) applications does bode well for industry success in replacing the publically offending surgical traditions of castration and spaying given the will to do so!!

In addition new control/contraception application tools also mean potential new ways to improve, tighten or better manage overall herd reproduction to best dovetail with the seasonal feed-base, reduce current severe wastage and poor performance eg out of wet season calving cows and heifers, and thereby greatly improve productivity through better reproduction control and management.

Contrariwise the best-fit win-win outcome, particularly in terms of tool free direct cost/benefit, may yet prove to be an industry paradigm shift towards young entire males being the animals of choice for a new finishing/marketing strategy. That would then leave new, more costly, tools to be applied largely to only the female herd, to deliver preferred time control of conception (and therefore calving/lactation) or sterility, in both heifers (replacement and surplus/cull) and the cull cow elements of the herd.

Literature Review circa May 2013

The literature review was facilitated by research access through “Summon” to the University of Queensland on-line library for the project author and the he gives credit and thanks to UQ for the access.

This allowed extensive key word searches to explore general and particular elements of the subject eg GnRH agonists (specific general search) with follow-up search for say Histrelin (one such agonist, result n=274 references) with subsequent appraisal and modified search for Histrelin in cattle, giving one reference for use in deer but no references for cattle per se. Details of the search plan are given in the Methodology section.

The literature review results will be summarised as follows:

- First a chronological listing (most recent to more dated) of relevant reviews with a brief description of their author/subject bias, depth and coverage and usefulness to the project review and for future reference by MLA staff and others eg researchers.
- Second a brief subject by subject summary and critique of technical learnings from the review as they inform the project goals and assist in making recommendations for any promising future short or long term R&D and assessing technical issues likely to influence success and/or failure of current or future projects
- Third to highlight relevant areas of particular knowledge for which a more detailed literature review by a practising scientist with expertise in the field would potentially add value to this review ie scoping studies for actual or likely new project investments.

Review documents have been collected electronically and/or in hard-copy for key references and will be filed at MLA and on the MLA data base as companion resource documents to the report for future referral by approved readers. They are not directly appended to the report itself.

A comprehensive bibliography or content summary from the individual reviews has NOT been repeated within this report. Rather relevant reviews are identified in full in the text for direct reference and any key research papers, referred to in detail in the review summary or in the recommendations section, are clearly cited in the additional concise bibliography, where a primary source is deemed necessary to verify the basis of discussion or recommendation.

Chronological Reviews relevant to Contraception in Animals/Man

2012 Formulation and delivery of vaccines: Ongoing challenges for animal management Sameer Sharma and Lyn A. Hinds J Pharm.Bioallied Sci 4(4) Oct.-Dec.258-266
134 references covers vaccines including immuno-contraceptive vaccines with discussion from oral to virally vectored delivery; formulation and delivery of vaccines for animals plus overall cost is seen as the current major limitations. This review is a usefully current analysis from Australian scientists in CSIRO Ecosystem Sciences.

2011 Review: Is there a role for immunocontraception? McLaughlin E.A. and Aitken R.J. Molecular and Cellular Endocrinology 335:78-88
150+ references form a review with a primary human contraception focus but broad scope, including other animals learning ie “we conclude that the specifications for a safe, effective, reversible vaccine are more likely to be met in animals than man”. This has proved to be a “go to” reference (and authors) from an Australian based group at Newcastle University, NSW with high quality, academic assessment of immunocontraception as a broad but specific field of endeavour. It is essential, most current, reading and critical to this project as the authors are active researchers in the field (See also in recommendations)

2011 Contraceptive Vaccines: Success, Status and Future Perspective - Guest Editorial pp 2-4 Rajesh K. Naz West Virginia University School of Medicine, Morgantown WV USA Am. J. Reprod. Immunol. 66:1-69 Editorial + 6 reviews on specialist areas.

Special issue of the AJRI claiming “a unique and comprehensive treatise offering up- to-date information on immune-contraception” including coverage in animals and man. There is a useful summary Table 1 p4 covering success and potential limitations across the range of targets for immunocontraception.
Sub-reviews are:
Antisperm Contraceptive Vaccines: Where we are and Where are we going? R.K.Naz pp 5-12, 54 references
Contraceptive Vaccines Targeting Factors Involved in Establishment of Pregnancy
Angela R. Lemons and R.K.Naz pp 13-25, 125 references
Immunological Approaches Against Human Chorionic Gonadotropin for Control of Fertility and Therapy of Advanced-Stage Cancers Expressing hCG/Subunits
Gursaran P. Talwar, Jagdish C. Gupta and Neha V. Shanker pp 26-39, 80 references

2011 Contraceptive Vaccines for Wildlife: A Review Jay F. Kirkpatrick, Robin O. Lyda and Kimberly M. Frank pp 40-50, 106 references
The review gives a comprehensive update on applications in up to 85 species for ZP antigens and GnRH vaccines. Detailed coverage and discussion of safety, efficacy, duration of action etc is presented leading to highlight of some issues for resolution to gain funding and general acceptance eg costs and risk(s) with virally vectored vaccines.

Contraceptive Vaccines Based on the Zona Pellucida Glycoprotein for Dogs and Other Wildlife Population Management Satish K.Gupta and 8 other authors pp51-62, 77 references
Another comprehensive review of Zona Pellucida science for contraception particularly in street dogs in the Indian sub-continent – includes multiple strategies to enhance vaccine efficacy including immunogen design and presentation including live vectored and DNA vaccine delivery approaches.

Contraceptive Vaccines for the Humane Control of Community Cat Populations
Julie K. Levy pp 63-70, 55 references
This review has a focus on GnRH vaccine in cats as Zona Pellucida vaccines are ineffective in cats –an apparent species difference. Gonicon® shows good efficacy but variable duration in cats (both female and male) but the cat appears to be intolerant of the current oil and Mycobacterial adjuvant mixture with discharging late onset granulomata evident post-vaccination in significant numbers of vaccinates. This highlights the need for species specific testing of any vaccine approach including antigen and formulation

2011 Review: Kisspeptin and fertility Saira Hameed, Channa N. Jayasena and Waljit S. Dhillo Journal of Endocrinology 208:97-105
Comprehensive review (approx.108 references) of the physiology of a new centrally acting peptide hormone family which is also found in some peripheral sites viz testis, ovary, anterior pituitary gonadotrophs, pancreas and small intestine plus particularly the human placenta (function unknown). Wide ranging effects on puberty, seasonal breeding, nutritional infertility, GnRH release and gonadotrophin(s) release are evidenced. Some work in sheep is cited eg seasonality but not cattle. Knowledge expansion is important.

2010 Review of issues concerning the use of reproductive inhibitors, with particular emphasis on resolving human-wildlife conflicts in North America Kathleen A. Fagerstone, Lowell A. Miller, Gary Killian and Christi A. Yoder Integrative Zoology 1:15-30 doi: 10.1111/j.1749-4877.2010.00185.x at http://digitalcommons.unl.edu/icwdm_usdanwrc
A broad ranging review (118 references) around direct chemical and indirect immunocontraceptive approaches to the diversity of avian and mammalian species in conflict with humans eg Canada geese, white tailed deer, pigeons and rodents etc. Technical strengths and weaknesses are addressed for each approach and applicability to various species. The value of the best delivery technology is interwoven in the discussion eg single shot zona pellucida vaccine for deer and horses. The regulatory, economic and health and safety issues are well canvassed in separate sections, together with public attitudes to wildlife fertility control This is a broad, very thoughtful and thought provoking review. The senior author (KAF) visited MLA Sydney in 2009 after the

Vertebrate Pests Conference in Darwin and provided a detailed briefing on USA research given MLA interest in Gonicon™ as a near market ready (for white tailed deer in USA) immune-contraceptive technology. This led to the proof of principle application projects in cattle – see MLA Project Reviews Section.

2009 Review: Gamete Immunology: Infertility and Contraception. Koji Koyama Reproductive Immunology and Biology 24(1):1-17 - 95 references
The immunology of infertility is critically examined in two sections namely anti-sperm anti-bodies in men and women followed by possible contraceptive vaccines using zona pellucida antigens based on the authors work. Ovarian failure/infertility is noted with ZP vaccines and discussed.

2009 Alliance for contraception in Cats and Dogs (ACC&D) Immunocontraceptive approaches for Sterilization of Dogs and Cats Scientific Think Tank November 19-21,2009 Roanoke, VA, USA pp1-12 at www.acc-d.org
A 12 scientist think tank process over 3 days with summarised findings for a final report and conclusions/recommendations (n=12) plus references (n=12, years 2002-2009) The focus was on reducing dog and cat over-population with preferred non-surgical approaches. Given science constraints an incremental approach was suggested plus a back to basics for routes to sterilisation. This is a useful resource for cross-fertilised ideas on a complex issue in the companion animal field for which tools are publicly demanded.

2008 Review: New perspectives in non-hormonal male contraception. Dolores D. Mruk Trends in Endocrinology and Metabolism 19(2):57-64
76 references support a human focused review from USA Population Council addressing potential for human male contraception. Table 1 comprehensively details then current drugs or targets. Non-hormonal is the key coverage. Review is a valuable recent back-ground up-date. Little vaccine content is included which is noteworthy ie the human male focus.

2008 Male contraceptive technology for nonhuman male mammals R.A. Bowen Colorado USA, Animal Reproduction Science 105:139-143.
Short review with 20 references focused on then current state of male contraception. A useful brief update mindful of the still to be resolved limitations of then new approaches eg vaccines/commercialisation, testicular injection/untoward sequelae, etc

2008 Review: Controlling Animal populations Using Anti-fertility Vaccines. R.Fayrer-Hosken Reprod. Dom. Anim. 43(Suppl.2):179-185
This review has a large animal perspective from USA College of Veterinary Medicine (Athens,GA.) author. Primary focus is zona pellucida and GnRH vaccines then already being developed for animal population control. 68 references

2006 Non-surgical methods of contraception and sterilization Michelle Kutzler and Anna Wood Theriogenology 66:514-525 108 references
USA Humane Society dog and cat focused review by Oregan State CVM, Corvallis Or. The review covers hormonal and non-hormonal topics with strong section on intra-gonadal injections in male dogs. It explores all possibilities practically including new products emerging for stray urban animal control.

2006 Mini-review: Biological control of vertebrate pests using virally vectored immunocontraception C.M. Hardy, L.A. Hinds + 5 others (CSIRO, Uni. WA). J. Reprod. Immunology 71:102-111
45 references in a proof of concept style review article. Zona pellucida 3 antigen gave sterility in mice when carried by ectromelia virus. However practical application to rabbits, foxes etc, is described as “remaining a daunting task”. No livestock applications described.

2005 Special Issue - GnRH in Domestic Animal Reproduction Edited by K. L. Macmillan and Jin-Gui Gong Animal Reproduction Science 88 (Issues 1-2):1-168 including 13 Section Reviews.

This is a huge and comprehensive compilation of the knowledge of Gonadotrophin Releasing Hormone (GnRH) by academics and industry players. It is essential specialist background reading on chemistry, synthesis, structure, receptors, regulation, effects in pituitary and non-hypothalamic reproductive tissues etc, etc. Sections 10-12 covers GnRH analogues ie agonists and antagonists and their roles in male and female fertility. These were key resources for this project as a platform for up-dating searches in the GnRH agonist/antagonist chemical contraceptive field.
Note Section 10 GnRH analogues- agonists and antagonists A. M. Padula pp 115-126
Section 11 Using GnRH and GnRH analogues to modulate testis function and enhance the productivity of domestic animals Thomas E.Adams pp 127-139
Section 12 Applications of GnRH in the control and management of fertility in female animals. C. A. Herbert and T. E. Trigg pp 141-153

1997 Hormonal and non-hormonal targets at implantation as targets for contraception Gui-Ying Nie, Anna R.Butt, Lois A. Salamonsen and Jock K. Findlay Reprod.Fertil.Dev.9:65-76
Early Australian approaches to implantation as a time critical control point for contraception. Based on molecular methods to identify new targets in mouse model and feral animal species eg Leukaemia inhibitory factor (LIF) identified in mouse. Useful as background but more recent information available (McLaughlin and Aitken 2011 Section 2.4 including marsupial studies ie CP4)

Subject by Subject Technical Content Summary and Critique re Contraception in Domestic Ruminants as Alternatives to Castration and Spaying (of cattle)

The aim here is to abstract reviewed literature content to assist in making recommendations for any promising future short or long term R&D initiatives and in assessing technical issues likely to influence success and/or failure of current or future projects. The focus is on production animals and of course those of interest to MLA levy payers in particular.

Introduction

Historically knife castration with forcible restraint but without anaesthesia in males and induced abortion in females was the shadowy status quo of contraception (neutering) for man or his chattels - slaves, domestic pets and animals. Natural fertility and disease/infertility were supreme and unfettered, with consequent high morbidity and mortality.

The advent of modern medicine/surgery eg sanitation, disinfectants, anti-biotics, vaccines etc, raised reproductive survival levels and fostered surgical contraception with its generally more direct and certain outcomes. Thus, dawned a more enlightened western cultural norm of castration and ovariectomy (spaying) in both animals and man.

Non-surgical approaches to contraception are entirely 20thCentury onwards, as the sciences around reproduction, endocrinology and immunology were unravelled and applied. Medical progress generally (particularly drugs/hormonal treatments including “the pill”) preceded that in animals, except for human assisted fertility ie AI, IVF, MOET etc where animal applications were models for human adaptation and thereby fostered social acceptance.

To-day contraception research is driven by past limitations in outcomes eg cost, reliability, etc, and by still pressing human population control needs world-wide. It is driven also by community aspirations for better management of surplus and nuisance animals (eg urban stray dogs and cats), or damaging animal populations (eg mice, foxes, white tailed deer (USA), Canada geese and pigeons etc,) in many countries. Thus conservationists, wildlife managers, urban officials, veterinarians and livestock/animal managers through to doctors and human population planners all confer across the contraception focused science agenda. This is strongly reflected in the literature cited above in the chronological review and its organisational origins.

However this shared comparative knowledge shows that biological variation even between mammal species is very significant and demands attention down to the level of establishing proof of principle in each target species and with each technology eg little success with ZP vaccines in cats (J K Levy 2011 review cited above),and GnRH agonists chronically administered in adult male cattle have enhanced, not suppressed, testosterone responses (D’Occhio et al 2000)

The following are specific subject based technical comments and summaries derived from the reviews.

Hormonal Contraceptives

The reviews by Bowen 2008 (male mammals), Kutzler and Wood 2006 (dogs and cats) and Fagerstone et al 2010 (for wildlife) have relevant sections. The former notes that excluding research with humans, a majority of the work on steroidal hormone contraception (of males) has been conducted in dogs, often as clinical test animals for human products.

Kutzler and Wood 2006 cover both progestins and androgens in male and female dogs and cats in some detail whilst prefacing this with the observation that “hormonal down-regulation is an alternative for TEMPORARY (my emphasis) suppression of fertility in breeding animals”.

Fagerstone et al cite various research applications of oestrogens and progestins in female wildlife and androgens in male rodents and wolves. In addition bisdiamine and alpha-chlorohydrin (male chemosterilants) were variably effective in gray wolves and rats.

However they conclude that “Although steroids can be fed orally or implanted they are effective for only a short period and need repetitive applications, making them costly and impractical in most field situations. Some, such as diethylstilbestrol, persist in tissue and in the food chain, making them unsatisfactory from an environmental point of view. They can also have deleterious health effects on treated animals.”

In regard to production animals, extensive review literature relevant to oestrous cycle control/manipulation over 1-60 days, ie around improving fertility, was noted in the searches but deliberately passed by entirely as irrelevant to this project. This is consistent with the above reviews on the temporary contraceptive effects of available chemicals. However it is NOT consistent with the industry specification developed (see Methodology) for a useful contraceptive, which must deliver long term infertility outcomes with minimal labour inputs and low cost. Ruminant clinical reproduction drugs (including steroid hormones) do NOT fit these dual criteria for low cost contraception over extended time frames.

However, an exception is the prostaglandins used for targeted early abortion post pregnancy diagnosis. They are specifically contradictory to this, in clinically effecting a not pregnant outcome by abortion after a point in time single dose, which may be seasonally beneficial in a whole herd context, particularly in extensive tropical northern beef herds. This approach is current practice by some private veterinarians thus providing an individual cow “delayed” contraceptive outcome and thereby better seasonally aligning and condensing the herd calving distribution. However productivity wise it is essentially inefficient in biological whole herd terms.

In addition modern food/meat production and exogenous “hormone” administration to animals in that production system which are near slaughter, is a market driven MUST NOT DO!! Therefore any technical possibilities for long term delivery of current products as depots would be cancelled on the drawing board and never considered commercially viable.

Finally Fagerstone et al 2010 has a small interesting section on chemicals that cause premature ovarian senescence, principally VCD (4-vinylcyclohexene diepoxide). It acts by depleting small pre-antral ovarian follicles. A treatment of10-30 days in rats and mice can produce almost complete follicular exhaustion and a menopausal-like state. This emphasises the potential for targeting germinal cells to effect sterility or (ir)reversible contraception – an approach discussed in the recommendations but using new immuno-contraceptive technology (McLaughlin and Aitken 2011).

Hormonal Down Regulation for Contraception

Gonadotrophin Releasing Hormone (GnRH) – Agonists and Antagonists
The most definitive review collated is Macmillan and Gong 2005, a special issue of Animal Reproduction Science with Sections 10-12, pp115-153 being highly applicable. Fagerstone et al 2010 (in wildlife) and Kutzler and Wood 2006 (in dogs and cats) provide species specific data, which re-enforces the wide general efficacy for contraception of GnRH agonists in particular, due to their central pituitary level effects.

The section 10 (Padula 2005) review starts with the peptide science of porcine GnRH elucidated in 1971 and proceeds to detail the peptide structure/function correlates of both agonists and later the antagonists, through to the 3rd generation antagonist Cetrorelix which has higher potency and no histamine releasing side effects –an earlier major handicap! All are synthetic analogues of GnRH.

The availability of all these bioactive peptides is driven by human use ie design, clinical application, demand and cost. Factors as divergent as demand for prostate cancer therapy and suitability for human IVF clinical use are at play. Suprelorin® is one deslorelin agonist formulation specifically developed for longer term use in domestic animals and is being commercialised world-wide (Peptech Animal Health Sydney Australia) It has high potency for preferred parenteral long term

continuous delivery, using mini-osmotic pumps, biocompatible cholesterol implants, polymer coated matrices, biodegradable microspheres etc in cattle as delivery system(s).

In the Macmillan and Gong (2005) review, the authors cover in sections11 (Adams T.E. pp 127-139) &12(Herbert and Trigg pp 141-153) male and female animal applications for agonists and antagonists.

A new search for GnRH and antagonists in cattle found n=225 references but little of value for contraception, except Jimenez-Severiano et al 2007 detailing in bulls and rams differing extended comparative responses using an antagonist and an agonist compared with concurrent control animals.

The Herbert and Trigg review also confirms evidence that agonist treated bulls have a paradoxical enhanced steroidogenic capacity (increased testis size, weight and testosterone secretion) following persistent exposure to agonist. This is clearly not contraceptive!! However rams appear to respond more conventionally (like dogs, rats and mice etc) with a transient stimulatory response followed by suppressed secretion of luteinising hormone (LH) and testosterone (Lincoln et al 1986).

Finally Junaidi 2007, in referring to ram studies in comparison with his male dog findings using deslorelin, points to the need to study each animal species, each agonist chemistry and each delivery mode eg daily injections vs constant infusion (and probably each sustained release bioimplant) combination to determine the net infertility outcome explicitly.

Herbert and Trigg 2005 finally assert that " While GnRH antagonists have the advantage that they do not provoke the initial stimulatory effect of agonists and their effects are immediate, the high dose (ie, lower relative potency) required to prevent oestrous cycles and the failure (therefore) to develop an efficient controlled release system has reduced their practicality." Thus overall cost clearly favours agonists and especially those of high potency such as deslorelin – a so called GnRH superagonist.

The review authors Herbert and Trigg 2005 Table 1 p143, also compared a range of agonists commercially available. To update the information this project reviewed agonists for cattle in general and those of similar potency to deslorelin, ie,triptorelin and histrelin for use specifically in cattle. The general review found n=149 references with no new applications of note in cattle or sheep (reference summaries available in hard copy) and no performance reports superior to that reported for deslorelin.

Histrelin is cited by Herbert and Trigg 2005 as almost twice as potent as deslorelin. A specific search revealed n=274 references mostly for application to human conditions eg prostate cancer etc and only one animal study in deer (Becker and Katz 1995) with none detected for cattle. A consideration of this superagonist for trial in cattle may be warranted if actual lower active commercial chemical costs (imputed from greater potency) are critical to a successful depot product outcome (see recommendations).

Finally leuprolide, a less potent agonist, when effectively formulated can deliver successful one year contraception in free-ranging elk in USA (Conner et al 2007) with consequent high reversibility. This demonstrates proof of principle in free ranging wild ungulates but at a significant cost, estimated at $150-$200 per dose in this paper.

This again highlights the potency, drug supply cost and formulation issues for successful field delivery. These are generally commercial in confidence matters and thus they emphasise the need for full pharmaceutical industry commercial engagement, assessment, development and co-operation with MLA and vice versa, if a viable product is to emerge for large animal use.

Finally therefore, if producers targeted up to but no more than one year of active use in lower body weight heifers only (to minimise total effective dose) this could become a critical component of

successful ie economical, product design. This focus would also target heifer productivity/time and season of first calving etc and utilise agonist reversibility synergistically to add value. Thus industry specification requirements should be quantified carefully ie body weight/rate of gain etc of target heifers, and be communicated to product developers early in the design stages.

Immunocontraception
Contraceptive vaccines have held sway as the likely technology of the future for only the last 20-30+ years, even though Baskin 1932 used human sperm injection to produce reversible sterilization in fertile women. This reflects the drug based development priority towards medical interventions and the parallel rise of steroid hormonal fertility control for individual women, accessible by them when resident in richer, better educated circumstances and in economies with reliable access to modern pharmaceuticals.

The three major, recent (2011(n=2) and 2010), reviews cited in this report draw attention to the continuing vision for vaccines but for humans, in particular, there is a focus on reasons for the lagging expectations and the absence of clinically applicable outcomes.

First the Australian review by McLaughlin and Aitken 2011 (Is there a role for immunocontraception?) draws attention in the abstract to world population growth, the paucity of the current contraceptive armoury, continuing abortion levels worldwide, developing country needs and the positive potential for safe, effective, easy to use contraceptive vaccines providing prolonged, reversible protection against pregnancy.

However they conclude that “the specifications for a safe, effective, reversible vaccine are more likely to be met in ANIMALS (my emphasis) than man”!!

This is a consequence in this reviewer’s judgement of stated or implied, almost utopian, medical specifications for humans for “fool-proof” contraceptive results eg100% efficacy, no hormonal perturbation (particularly for men), immediate temporal effect and total, at choice, reversibility!! No wonder the world population continues to grow, particularly in the developing world!

The second multi-part/ multi-author review (Contraceptive Vaccines: Success, Status and Future Perspective) edited by USA Professor of Obstetrics and Gynecology Dr Rajesh K Naz was published in the American Journal of Reproductive Immunology volume 66 in 2011. It suggests that “Contraceptive vaccines have been proposed as valuable alternatives that can fulfil most, if not all of the properties of an ideal contraceptive.” This is based on their high target specificity, long-term action, low cost, lack of side effects and utility and likely use in developing and developed countries with infrastructure for mass vaccinations.

However Table 1 p4 is an interesting success and potential limitations tabulation of vaccine targets. Only hCG vaccine has been developed for use in women but it is difficult to achieve high anti-body titres, though it has been clinically trialled successfully in India (Talwar et al 1994). Novel refinement/recombinant vaccines eg hCG beta-LTB, are in place and being trialled for bladder cancer patients (see sub-review Talwar et al 2011 Am.J.Reprod.Immunol.66:26-39) but contraceptive application for preventive human medicine lags more slowly (sadly) behind!!

In contrast GnRH and zona pellucida (ZP) glycoprotein antigens have begun to be actively commercially produced and applied in animals, both domestic and wild. However, they are discounted for human use because of the hormonal impotency consequences with GnRH and probable, but unproven (in humans), ovarian pathology with possible irreversibility (in part or completely) using ZP antigens. Thus the proven and deliverable scientific possibilities in animals of the last 2-3 decades can find no acceptable place in human population control, despite the urgent global need.

The third up to date review most relied upon for this report is that of Fagerstone et al 2010 in Integrative Zoology 1:15-30 entitled “Review of issues concerning the use of reproductive inhibitors, with particular emphasis on resolving human-wildlife conflicts in North America.” It

reviews current technology for wildlife contraception but then looks thoughtfully at adoption for use issues, such as regulation, biological and economic feasibility, economic practicality, health and safety issues and public attitudes towards wildlife fertility control. All of these have critical bearing on socio-economic acceptance in each human demographic and therefore implementation of contraception even for wildlife!!

Fagerstone et al 2010 discuss on page 24 the issue of hunting and tradition vs contraception for wild population control. Even biologists and wildlife agencies have shown reluctance!! Only 9% of relevant agencies/organisations (n=134) in the entire USA in 1994 had an established policy on wildlife contraception and this compared with just 39% of (n=54) environmental and animal activist groups (Sanborn et al 1994)!

These three reviews taken together detail the two key proven front-runners in the animal immunocontraception field.

These are first the zona pellucida (ZP) vaccines, targeting only females, and based on the glycoprotein layer located on the outer surface of the egg. Antibodies to ZP proteins result in infertility either by blocking sperm from penetrating the ZP layer or by interfering with egg maturation within the follicle (Dunbar and Schwoebel 1988) Porcine ZP (PZP) has been successfully tested in numerous species from dogs to baboons and wild horses – see Fagerstone et al 2010 review and Kirkpatrick et al 2011 sub-review.

PZP alone is not effective in cats or rodents. However at Murdoch University WA Eade et al 2009 studied vaccination of cats with PZP and with homologous feline ZP sub-unit (A and B+C) DNA vectors. The latter did elicit contraceptive effect with natural mating ie 20-25% vs 83% pregnant in treatment groups vs controls. The authors conclude that feline ZP sub-units are potential candidate antigens for immunocontraception. This demonstrates the advances possible with recent technical innovation. Rabbit ZP glycoproteins were similarly explored as antigens using a DNA vaccine delivery approach to evaluate immunocontraception in cattle with indications of success ie reduced estrous activity/ovarian function (Foley et al 2007).

McLaughlin and Aitken 2011 review this field and cite the induced ovarian pathology as a contra-indication for human use ie permanent ovarian damage due to immunodominant epitopes. However animal/wildlife applications are not necessarily inhibited by this constraint and practical outcomes can still be achieved (Kirkpatrick et al 2009).Indeed the high efficacy in many species, minor behavioural effects, the ovary specific action and impressive safety, even in pregnant females are strong arguments for zona pellucida based vaccines.

However, Kirkpatrick et al 2009 show that the detailed response of individual species needs to be explored to understand and then later effect a practical outcome ie sheep and goats exhibit high and prolonged ZP antibody titres and sheep exhibit significant ovarian disruption (Stoops et al 2006). Further this species exploration then also needs to be juxtaposed with industry need and specification for (permanent or otherwise) female contraception.

Taking female sheep/goats as the example of a likely responsive species, there seems to be no industry demand at all for contraception as females are either sold as unjoined lambs, joined as lambs or hoggets and the non-pregnant ewes culled or they enter the adult breeder flock. Ram control and management, especially of flock reproduction, is much easier compared with cattle and marketing decisions eg cull and finish, are readily achievable with short term supplementation.

In contrast cattle production is on a significantly longer reproductive, growth rate and seasonal production cycle/timeline and hence contraception for unwanted females emerges as a desirable management capacity. This being so the small body of research published on ZP contraception in cattle warrants further investigation particularly in the Australian northern cattle industry context – see Peripheral Female Reproduction Targets recommendations section.

Finally there are published reports of single dose long-term PZP vaccine success in seals (Brown et al 1997) and deer (Fraker et al 2002) using liposome encapsulation and Freund's Complete Adjuvant (SpayVac™). This is a major logistic advantage for wildlife contraception. The development of a technical solution for delivery of a long-term response in cattle awaits a more focused research effort given the limited studies to date. A DNA vaccination approach may be useful given its capacity to promote both typical B and T cell responses in the target host and the potential for transdermal administration (Foley et al 2007, Gupta et al 2011 sub-review Am. J. Reprod. Immunology 66:51-62)

The second major area of vaccinology is the **GnRH based immunocontraceptive vaccines**. These vaccines take advantage of the role played by GnRH in higher central or "upstream" regulation of mammalian reproduction.

Centrally GnRH, released from the hypothalamus in a pulsatile secretion pattern controls steroidogenesis and gametogenesis by stimulating the release of reproductive hormones from gonadotrophic cells in the pituitary. This triggers the cascade of reproductive hormones (FSH and LH in both males and females) that lead to all downstream testicular and ovarian functions.

Thus GnRH vaccines, when effectively blocking GnRH activity, have widespread effects on both males and females, with major behavioural (hormonal) effects and gonadal germ cell infertility. These outcomes mimic castration and ovariectomy but are reversible in the short term as antibody to GnRH declines. However reversibility in breeder females has not been tested long term after multiple boosters/years. McLaughlin and Aitkien 2011 emphasise these diverse effects to conclude the unsuitability of GnRH vaccines for human clinical use. In contrast Levy Julie K., 2011 –see sub-review Am. J. Reprod. Immunology 66:63-70 – is very positive about the potential for GnRH vaccine use in problem cat populations where modified sexual behaviour is a desirable bonus!!

In addition species in which the corpus luteum of pregnancy is supported by pituitary luteinising hormone (LH) will abort if treated with an anti-GnRH vaccine during pregnancy eg bovids. Thus careful individual vaccine treatment records over longer time spans would be essential to monitor both effective vaccination initially and later risk of pregnancy loss in any lapsed GnRH vaccinate which has conceived again. Pregnancy testing would be necessary to avoid unintentional losses prior to any booster round required, especially given the level of low or non-responders (possibly up to 50%) which is high-lighted as an important area of short-coming by earlier reviewers (Herbert and Trigg 2005 –see chronological reviews section or Anim. Reprod. Science 88: 141-153.)

Further still GnRH receptors in other tissues and other GnRH effects within the CNS eg on cardiac function, are associated with side-effects in man and possibly therefore other animals –see Kirkpatrick et al sub-review p43 and Table 1 p46.

This conflicting balance of pros and cons has not prevented commercial development of suitable GnRH/antigen complexes (to make the small GnRH molecule immunogenic) combined with a range of adjuvants to augment the antibody response to a sufficient level to achieve contraception. These combinations have been tailored to achieve particular development goals eg long term response in both female and sometimes male wildlife with Gonicon™ (Miller 2004, Fagerstone et al 2010 review).

In the grazing production animal field the commercial focus to date for vaccine applications has been on marketing of grown out young adult bulls (Amatayakul-Chantler et al, 2013, Amatayakul-Chantler et al 2012) or on behavioural modifications from hormonal suppression in males and/or females in short term contexts or management niches eg feedlots (estrous suppression, Charman 2013, onset of puberty/pregnancy prevention, Geary 2006) or on winter pastures for dairy bulls in New Zealand using Bopriva®. These particular short-term applications reflect the likely successful potential product applications and the actual label claims!

However the study by Janett et al 2012 using the GnRH vaccine Bopriva® in prepubertal bull calves, whilst successful in decreasing testosterone concentrations and delaying puberty for some 10 weeks (mean delay), also demonstrates the challenges for this GnRH approach in achieving complete non-surgical castration.

This reflects the variable immunological nature of this technology approach and suggests new avenues may be required to fully achieve the welfare focused goals of true alternatives to castration and spaying. The recommendations around kisspeptins and GnRH toxin conjugates are driven by the discussed present limitations and are focused on newer scientific possibilities which may help provide the future alternatives.

The refinement of GnRH vaccines, particularly the upgraded quantum of antibody response and duration should not be abandoned prematurely but should be the R&D preserve of commercial investment (perhaps supported by MLA donor Company) to facilitate the best product deliverable technically possible for industry.

Physico-chemical Approaches to Contraception

These approaches are evident within the review articles cited and are appraised briefly here for completeness, whilst noting overall that they do not yet, in my view, constitute a preferred, genuinely superior and welfare friendly replacement for the surgical (castration and spaying) procedures.

The reviews cited in the text of this report by Bowen R A 2008 and Kutzler and Wood 2006 are relevant. They review older applications research particularly with respect to contraception by intra-testicular injection of necrotising/sterilising chemicals in male dogs for urban animal control. Bowen draws attention to the consequent possible untoward sequelae when chemical injection is used widely and /or inexpertly - later surgical castration being then required as a remedy to restore the welfare of the individual dog. See also Levy Julie K. et al 2008 using zinc gluconate compared with Jana and Samanta 2007 using Calcium chloride.

The same technique has been applied to albino rats, tom cats, goats and even male cattle (Canpolat I et al 2006) but generally has not been whole-heartedly or widely adopted, even in the USA, despite a commercial product “Neutersol®” Injectable Solution for Dogs being marketed in that country. However Levy et al 2008 and Jana and Samanta 2007 both argue for applications for male dogs in remote locations or less developed communities.

Project P.AWW.0219 – Chemical Sterilisation as an Alternative to Spaying of Heifers. This MLA project has commenced funding in 2013 concurrent with the start of this review. The application contains a brief but useful literature review of the use of chemicals for contraceptive purposes (predominantly in males), whilst noting that the technique has not been tested in female cattle.

This reviewer’s comments critiquing these approaches are found in the section of this report dealing with current levy funded projects in the MLA Current and Retrospective Projects Review section. Overall these techniques (for application to either gender) do not deliver an outcome which meets desirable industry specifications, including improved productivity. Particularly they are significantly more labour intensive and therefore time consuming and costly, including in terms of additional materials or veterinary expertise. Thus they are not an owner/operator driven crush-side replacement solution.

Intra-uterine devices

These devices were a commercially designed adaptation for the bovine of the now largely discredited/discarded human IUD technology used initially in many countries world-wide.

An Argentine study (Turin et al 1997) in Bos Taurus cattle reported remarkably good oestrous suppression (98%) and contraception (100%) in 220 heifers, plus additional weight gain. None were pregnant after a 120 day natural joining period.

In stark contrast a study in Brahman cattle in Australia (Fordyce et al 2001) concluded as follows: “Because of the difficulties of implanting bovine IUDs, the high frequency of associated uterine injury, the high pregnancy rate in implanted animals, and that growth was unaffected by the presence of a bovine IUD, it was concluded that the device had poor contraception efficacy and no growth-promoting effect in Brahman cattle.”

The devices are no longer commercially available, nor advocated in Australia.

Canvas Academic Expertise

The academic and industry colleague interactions contributing to this review are described in the Methodology section. This included the initial scoping meeting in Brisbane, several one on one interviews and extensive email contact with Australian scientists and published authors from overseas eg Professor Terry Nett, Colorado USA.

Drs Norman and Chenoweth from Charles Sturt University kindly gave access to their pre-publication book Chapter “Male Animal Contraception” for cross-reference. Dr Norman was unable to be interviewed during the research period for personal family reasons.

One on one meeting(s) were held with Dr Michael K Holland CSIRO, Professor John Aitkin and Dr Eileen A McLaughlin from University of Newcastle NSW.

All these interactions are most evident and are incorporated in the directions and ideas for recommendations from the project which make up the concluding elements of this report. I freely acknowledge these valuable contributions.

Concluding Recommendations

These recommendations will scope technical areas in the broad field of contraception for potential future MLA investment within the 2012 -2016 Animal Welfare Strategic Plan. They will include project modifications/extensions plus some new projects and indeed proposals for entire new research areas of basic and more applied research.

They are informed by the project literature review and expert professional and scientific input to the review process.

The aim of the recommendations is to inform and help focus and direct future MLA Animal Welfare and related investments over the next 4-5 years. Specifically they will seek to deliver to industry as soon as possible alternative tools and thence outcomes, which will allow cattle and sheep/goat producers to sustainably and progressively achieve a better industry position re welfare risk ie less castration of post-juvenile animals, especially those over 6 -12 months of age and less cattle spaying.

MLA management will thereafter independently determine all investment priorities, projects and schedules etc whether based on these recommendations in part or whole or any other related or independent considerations.

Recommendations are presented under the topic headings covered in the literature review with positive alternatives primarily being presented. For example GnRH antagonists are omitted as the review found that low relative potency and high drug cost was a major constraint for human clinical use, let alone animal applications. Also there were limited technologies eg drugs or bio- functional advantages compared with GnRH agonists.

Similarly IUDs and chemical castration will not be further considered.

Recommendations by Subject Area

Hormonal Down Regulation

Progestins/Androgens

It was commended to this reviewer that the potential for the contraceptive use of long-acting, slow release progesterone implants be explored with commercial interests as this technology is readily available eg in dogs (Kutzler and Woods 2006).

Indeed in cattle much current short-term use is made of these and many other hormonal products which are readily available for multiple programs delivering manipulation of the oestrus cycle for improved fertility, including fixed time artificial insemination, MOET etc.

However the repeated or long-term (slow release) use of steroid hormones in food producing animals for long-term contraception is strongly contra-indicated, in principle, from a marketing and food safety perspective. In addition any significantly lower cost-structures for products suitable for long-term administration is considered commercially improbable! Thus no recommendation has been brought forward here. The same logic applies to androgens and now even (unscientifically) to other growth promotants.

GnRH agonists

GnRH agonists can be effectively used to prevent ovulation longer term in female cattle while in adult male cattle (but not rams) they contradictorily have a stimulatory effect on gonadal testosterone activity (D'Occhio et al 2000 and 2002).Thus the chronic inhibition of the pituitary-gonadal axis in heifers/cows ultimately suppresses oestrus and delivers effective long-term dose dependent contraception.

GnRH agonists such as Deslorelin (Superlorelin®) with high potency and longer half-life, have been much researched and numerous synthetic analogues (Herbert and Trigg 2005 review,

Table1&2) developed and applied in a range of mammal species. This together with various slow release/bio-implant technologies has allowed commercial contraceptive success, including in cattle(D'Occhio et al 2002). These animal applications follow long standing and widespread human hormonal-dependent reproductive and neoplastic disease applications eg in precocious puberty, endometriosis, uterine fibroids, breast and prostate cancer.

Contraception in females is effected after a single depot injection without the need for use of adjuvants and with extremely high contraceptive success rate. Reversibility is retained and can be stimulated by FSH administration. These are key positive specifications, particularly for replacement heifer reproduction control and management.

Recommendations are as follows:

Encourage current commercial investor(s) to examine/utilise all available cost effective synthetic high potency agonist formulations in their Australian product development program. Specifically consider histrelin which is twice as potent as deslorelin but which does NOT appear from the literature review to have been trialled in female cattle to date, only deer (Becker and Katz 1995).

Encourage current commercial investor(s) to research the use of their preferred least cost agonist preparation(s) in rams also. This follows early work by Lincoln et al (1986) which evidences a contraceptive response in rams. This work has not been followed up commercially to date to design an effective agonist/species/mode of delivery package. However industry has a now defined welfare related problem (S Barber pers comm.) of younger but pubertal rams which are excess to seed stock producers requirements for a number of reasons and for which castration, then sale for slaughter, is their preferred outcome. Effective non-surgical castration is a potentially valued and preferred technology in this specific seed stock commercial context.

Seek competitive, commercial, agonist technology via MLA Donor Company bid(s) using alternative agonist/bio-implant technology proprietarily owned by Peptech Australia P/L. This could generate genuine price competition which is desirable in the bovine GnRH agonist contraceptive marketplace.

Fund, using MLA levy funds, Producer Demonstration Site(s) or Research Station based multi-year applied R&D project(s) to optimise annual strategies for management of cow/heifer reproduction, using specific commercial GnRH agonist implant contraceptive technologies to manipulate and control the annual calving patterns. This should include cost/benefit analysis of interventions in heifers (to be joined as 1 year olds or delayed to 2 year old joining) and late and non-pregnant older or cull cows. The implant delivery specification dose/response dynamic should be critically appraised to inform producer adoption at the defined part herd level eg cull heifers to fatten/sell as non-pregnant after say 9 months contraception. Reproduction and marketing outcomes should be evaluated preferably across differing seasons. A "best practice" paradigm using agonist technology at defined/least(?) cost should be targeted.

These agonist recommendations should be prioritised by MLA for industry outcome delivery as agonists have a higher efficacy and more uniform biological success rate and less negative side effects than GnRH vaccination, where "poor or non-responders" in females may become an issue at the population/herd level.

The key remaining risk issue, even with GnRH agonists of high potency, is final product cost. Industry cost/benefit value perception will be critical to adoption in this space. Hence the recommendation for best practice/defined cost studies above in female cattle and potentially also in rams. This will help producers properly evaluate total cost/benefit for their business after proper appreciation of specific agonist technology performance criteria.

GnRH based Immuno-contraception

GnRH is a low molecular weight peptide hormone which needs to be conjugated to a large carrier protein in order to elicit an immune response. Immunisation against GnRH was originally used to better understand the role of GnRH as a higher level (hypothalamus and pituitary) "upstream" control of mammalian reproduction. Thereafter therapeutic applications evolved. Active immuno-contraception has been investigated in a range of mammals for over 20 years (See review Table 3, P147 by Herbert and Trigg 2005).The anti-GnRH response delivers both infertility and gonadal steroid hormone suppression.

However, these authors also identified the following practical issues likely affecting commercial application including:

- Highly variable anti-GnRH antibody response between individuals
- Highly variable anti-GnRH antibody response over extended time frames >6 months
- The emergence of "non-responders" in the population, probably genetic in origin
- Consequential genetic selection for non-responders from high level/frequency of use such as in breeding animals over multiple years
- Need currently for at least two immunisations to better achieve high anti-GnRH titres
- The need for strong adjuvants often with adverse injection site reactions
- OH&S risks of human self-inoculation.

Indeed these issues, particularly the need for at least two vaccinations, are identified by Herbert and Trigg 2005 as causal in the withdrawal of the cattle vaccine, Vaxstrate®, from the Australian and world market in the late 1990's.

Newer GnRH vaccination approaches (2000-2010) are reviewed by McLaughlin and Aitken 2011 as to feasibility and vaccine design but no studies are reported in cattle specifically but several in ram lambs. However Price et al 2003 did use a Gonicon-like vaccine successfully to reduce aggressive behaviour in bulls. After vaccination at 4 months of age and again at 12 months, males were found to have behaviours similar to that of steers. Additional to this, work by Pfizer Animal Health P/Ltd Australia (now Zoetis P/Ltd) is reported by Janett et al 2012 in male calves and in 2-3 year old bulls prior to slaughter in Brazil and Mexico (Amatayakul-Chantler et al 2013 and Amatayakul-Chantler et al 2012). This product (Bopriva) was commercially first released for use in bull behaviour modification in New Zealand and then in Australian feedlot heifers in 2012 to provide oestrus suppression for up to 10 weeks (Charman 2013). It appears to perform with reasonable efficacy in defined short term (up to 6 months) applications. However efficacy has been so far poorly defined by limited testosterone measures over time, especially during the decline phase, days 105-279 (Amatayakul-Chantler 2013).

In addition the review by McLaughlin and Aitken 2011 draws attention to the longevity of the contraceptive response in white tailed deer ie up to 5 years, derived from use of Gonacon™ and Gonacon-B™ and the AduVac™ adjuvant formulation, containing M. avium in particular. Thus the possibility for duration of contraceptive effect with median of 12 months or longer clearly exists but delivery requires specific solution of product formulation issues, particularly the adjuvant and/or slow release components.

Recommendations are as follows:

A key issue is the priming + second vaccination interval (at up to 60+ days apart with females not actively contracepted in the interim) for generating long-term high anti-GnRH anti-body response and the key determining role in that interval metric of the chosen adjuvant/immunogen combination. The other flip side is whether the chosen technology can deliver any effective "one shot" response, particularly in cattle, which appear to be slow immune responders. Finally the non-pregnant contraceptive outcome and herd profile (non-pregnant vs if pregnant and when?) are both critical outcome measures in female cattle.

Compare critically GnRH immuno-contraceptive approaches to allow discriminating investment judgements in general (about the GnRH based immuno-contraceptive approach vs the agonist approach) and in particular about further Gonicon™ related investments for use in cattle (female and male) or rams. This is important given the Gonicon™ product pathway to commercialisation is currently unclear, as is the single shot response in cattle. If performance vs specification criteria are poor eg unknown or poor efficacy figures beyond 180 – 400+ days, reliance on one or more booster vaccinations etc, then do NOT support commercialisation, unless the lack can resolved by critical strategic scientific modifications!

Given the current absent or unclear commercialisation pathway, critically examine likely avenues for commercial availability of Gonicon™ to Australian livestock industries if it meets industry outcome specifications. Clarify with the Invasive Animals CRC (IACRC) any product licensing agreements they may currently have with USDA for import of product. Examine options for local manufacture and supply as an approved alternative for livestock, requiring APVMA registration and in possible collaboration with the IACRC (using non-registered(?) product for wildlife?)

In the light of the investigations above, and of the future results of the new male Gonicon™ castration project recently (2013) approved, critically re-appraise the prospects for the Gonicon™ related investments in general eg likely pathway to commercialisation, commercial competition, non-responder rate, degree/duration of castration effect, reversibility, one shot efficacy etc. Also re-assess the key performance criteria around sero-cross reactivity with BJD (Bovine Johnes Disease) and whether a “one shot” contraceptive response is biologically achievable in cattle (male or female) with Gonicon™. Thereafter further proof of principle studies MAY be indicated with Gonicon™ to deliver clarity eg, immunogen dose rate in heifers/cows, adjuvant ± M.avium in cattle, quantum of immune response etc, for the explicit purposes of a stop/go decision vis a vis the use of this wild-life (white tailed deer USA) designed product in cattle. (See also Gonicon™ current project review section above re externality issues)

If two or more vaccinations are still required in female cattle the project may NOT meet industry specifications, unless a bridging technology eg GnRH agonist can economically effect contraception for the time period between priming and booster vaccinations.

MLA should consider within project adoption of, or future investment alternatives in, GnRH vaccine refinement technologies including:

In sheep, alternative coupling proteins and adjuvant components (Earl et al., 2006)

In cattle, alternative recombinant GnRH fusion protein production (Cook et al. 2000)
A DNA vaccine approach as reported in mice using plasmid encoding GnRH-I and T-helper epitopes (Khan et al., 2007) but noting the cautions on use of such vaccines by Babiuk and Babiuk, 2008.

Totally synthetic peptide based vaccines with novel T-helper cell epitopes –see in dogs (Walker et al 2007)

Better, safer novel adjuvants – see for GnRH vaccines, Section 2.1 pp79-80 McLaughlin and Aitken 2011

These alternatives should be actively considered, especially if GnRH vaccine remains the sole effective and preferred approach in the male bovine by which to attempt to replace surgical castration entirely, ie combining as it does contraception and hormonal neutering. This suggestion notes the contrary effect of GnRH chemical agonist treatment in post-pubertal bulls thus decreasing other options for alternatives to castration.

GnRH Toxin Conjugates
This technology is also reviewed briefly in concept and the then limited practice, by the review Herbert and Trigg 2005. Subsequent to that time MLA made a modest early investment with University of Qld (Professor M. D'Occhio) in collaboration with Professor Terry Nett at Colorado State University USA to develop the technology and test it in rams, as a proof of principle in large production animals of interest to Australian industry. Unfortunately progress was so slow due to technical hurdles, that the final preferred product (GnRH conjugated with recombinant PAP) in large volume was not available for testing in Australia. It is recommended that this approach be the subject of rekindled negotiations for new investment on behalf of industry based on May/June 2013 email correspondence with Professor Nett et al. The critical appraisal, as part of contract negotiations, should include confidential MLA scientific review (perhaps face to face) of the now completed mouse studies conducted by the biotech venture Company and Colorado State University in the USA. Due diligence on the commercial prospects of the biotech venturer would also be prudent before any contract completion.

The KPIs should include indicative costs and production efficiency of the current recombinant derived product/production system and its quality assurance specifications, together with the state of the evidence for effective ablation of the pituitary gonadotrophe cells in vivo. The rationale is to seek evidence for a key desired outcome of long-term induced sterility in both male and female production animals with opportunity for industry to utilise the approach in all steers (replace castration) and all cull females (replace spaying).

The focus should be on male animals initially as other current technologies (GnRH agonists and GnRH vaccines) either do NOT work (agonists) or appear to work more poorly in terms of efficacy (50-70% with current vaccines) than castration per se. Thus the ablation concept and its effective in-vivo performance is critical and needs to be tested in production ruminants as early publications in male dogs suggested evidence of recovery/ reversibility after 20 weeks post-treatment (Sabeur et al., 2003). However the "product" has been modified since that time.

Further if ablation was significantly effective this product could potentially be used in near marketable 2 year old bulls to quickly reverse male sex characteristics and thus help achieve target grid and MSA specifications eg fat cover, after harvesting the growth rate benefits of bull beef production during the growth phase. This finishing outcome might also be achieved even direct from pasture, rather than via a feedlot finishing phase.

What controls the controller ie GnRH? – Prospects for "upstream" central control.
An answer was discovered in 1996 by D.K.Lee and colleagues (cited in the review by Hameed et al 2011 Journal of Endocrinology 208:97-105) – it is kisspeptins and the kisspeptin receptor. Peripherally the human placenta and centrally the rodent hypothalamus express high levels of kisspeptin proteins. Indeed this reviewers literature search for kisspeptins and fertility found n=1856 references. A first up review article (Hameed et al. 2011) documents roles for kisspeptin(s) as follows;

Stimulation of the release of GnRH through action at the kisspeptin receptor

Consequential stimulation of gonadotrophin release

A probable direct effect on the gonadotrophs of the anterior pituitary gland thus also stimulating directly the release of LH and FSH

A key role in mediating gonadal steroid feedback to the hypothalamus

Influence in the onset of puberty

Are implicated in seasonal breeding in hamsters and sheep (Clarke et al 2009)

Hypothalamic kisspeptin provides a critical direct link between nutritional status (body condition) and fertility

Exert an essential role in the generation of the LH surge for ovulation

Hameed et al. 2011 suggest kisspeptin therapy might stimulate a more natural pattern of reproductive hormone release and therefore better therapeutic outcomes eg in human IVF less risk of ovarian hyperstimulation syndrome currently associated with exogenous gonadotrophin injections.

Finally as a peptide hormone family the kisspeptins have already had monoclonal antibodies raised against them in rats with experimental direct bio-activity on pre-optic area GnRH cell bodies (neurones) leading to the complete blockade of the pre-ovulatory LH surge in female rats (Kinoshita et al 2005)

All this suggests significant potential for blocking or contraceptive interventions and possible "up-stream" or central hormonal manipulations with broad sterility consequences, generating thereby "central castrates" ie replacement for surgical castration in particular and spaying for cattle females surplus to breeder requirements.

Recommendations
MLA should seek strategic science investments in cattle and possibly sheep (as an experimental model) neuro-reproductive physiology which seek to exploit for contraceptive purposes the potential around new knowledge of higher hypothalamic pituitary axis functions such as kisspeptins or related neurobiological activities.

A kisspeptin peptide immuno-contraceptive or targeted toxin approach and/or kisspeptin agonists/antagonists etc should be explored. This might be combined with GnRH technologies (including GnRH toxin conjugates) targeting multi-functional disablement of the neurophysiologic drivers of puberty and reproduction. An initial scoping study is indicated. Dr Scott Norman CSU Wagga Wagga is one interested veterinary scientist/andrologist active in the field, who may wish to be engaged or to facilitate the proposed study. However MLA may prefer a national or international tender process.

Immuno-contraception targeting Gonads or other peripheral Reproductive Organs

Multiple components of either the male and/or female peripheral reproductive organs have been targeted in pursuit of the contraceptive outcome (McLaughlan and Aitken, 2011). Sperm have long been known to be highly immunogenic in both females and males and that women could be immunised using whole semen from their partners resulting in prolonged infertility (Baskin, 1932).

Peripheral Female Reproduction Targets for Immuno-contraception

The most widely explored candidate target for immuno-contraceptive vaccines in a wide range of species is the female egg zona pellucida glycoprotein complex with some 30+ years history (see review Frayer-Hosken 2008). Whole native porcine zona pelluida (PZP) harvested from pig ovaries at abattoirs is the most used antigen source (McLaughlan and Aitken 2011) but may be variably impure and requires standardisation and quality control for effective reliable use (Kirkpatrick et al. 2009) even in wildlife. Both references cited here very well summarise the issues from the human and wildlife perspectives, but it is noteworthy that little has been published in domestic cattle (Kirkpatrick et al 2009 cites success in yak, banteng and Bos taurus) and there is known to be considerable between species variability in response. However a 2007 study in Holstein cows showed some contraceptive efficacy using a rabbit ZP DNA vaccine approach (Foley et al 2007). Poor reversibility and variable ovarian pathology (significant in sheep - Stoops et al 2006) indicate a need to carefully evaluate zona pellucida antigens in each targeted species particularly cattle.

Recommendations

MLA should further examine in confidence the work currently being conducted independently by CSIRO and UQ (Drs Holland and McGowan) using pZP in cattle to establish proof of principle in the bovine and to evaluate the degree of ovarian pathology/sterility or potential reversibility. Their project is also exploring methods of delivery and adjuvants for generating a long-acting response, cognisant of the work of Turner et al., 2008 and Brown et al, 1997.

MLA should consider levy funding support for further refinement work, as required, in larger numbers of cattle to generate better baseline performance specifications and application data for any potential commercial development partner to consider.

The rational basis for proceeding would be a product specification targeted only at female cattle (heifers and/or cows) with non-reversible contraception, effective (100% if possible) with duration a minimum of 12 -24months after one priming plus one booster dose. It should be safe to use for animal and operator and easy to administer subcutaneously anywhere forward of the shoulder but preferably at or near the base of the ear.

Commercial investment for product development/registration should be sought as soon as possible. Additionally and very desirably a one shot or pulsed/extended release adjuvant and antigen delivery technology should be developed and employed to meet practical industry labour minimisation needs and the delivery of rapid reproductive control (Fraker et al. 2002), once empty cull females are identified.

This technology (not applicable to males) would need to look for market size outside Northern Australia alone and with the above specification could readily be utilised by beef producers in southern production systems for surplus, cull or fattening only classes of female (eg feedlot heifers and post-calving aged cows). A high effectiveness rate would greatly help generate interest, given capacity for safe application also to pregnant females (Kirkpatrick et al 2009) thus delivering post-calving sterility.

Commercial interest may be able to be delivered by a small business model given the pZP production is a natural product harvesting method NOT a synthetic manufactured product.

Marketability for meat production should be readily retained as there are no steroid hormone consequences and phenotype is unchanged.

This product type may find industry market preference to GnRH vaccines from a human safety perspective (jillaroos only not to use!) and because the non-responder issue, more prominent with GnRH vaccine, will be diminished or irrelevant.

Other peripheral female reproduction targets

McLaughlin and Aitken 2011 in a section 2.4, p82 explore a range of ovarian and endometrial targets as potential alternative immune-contraceptive antigens in a number of species but few are explored in depth beyond the mouse model. Vaccines targeted at the uterine endometrium, though non-hormonal, do not immediately impress as superior, through their post-conception mechanism of action and given the present state of knowledge. However they could potentially address a sterility approach for cull heifers and cows.

Indeed, this anti-implantation approach should not be assumed to be crudely abortifacient (as with IUDs) and therefore ineffective but perhaps the converse, given the known vulnerability of ruminants to early embryonic loss eg from transient concurrent pestiviraemia or post- MOET recipient implantation rates for example. A range of female large animal applications could potentially be considered, eg related to the cytokine interleukin 11(IL11) antagonists – see Menkhorst et al 2009.

Correspondence by the reviewer was initiated with McNatty et al., 2006 and 2007 in New Zealand re BMP15 and GDF9 claimed to have ovarian focused contraceptive potential in sheep, but no response was received. This suggests Australian scientific expertise is most likely to hear, understand and be responsive to expressed needs of Australian industry and its R&D funding aspirations.

Further, direct discussions with Professors R.J(John) Aitken and Eileen A. McLaughlin at the School of Environmental and Life Sciences, University of Newcastle, Callaghan NSW 2308 on 6th June 2013 elucidated that they have been actively (2010 to date) developing a broad gonadal germ cell contraceptive strategy for humans and potentially animals. I quote them as follows "We have developed and tested peptide-based reagents with binding specificities for germ cells. We have successfully used random phage peptide display libraries to derive these reagents from gonadal cells. The power of the phage peptide display library approach lies in its ability to discover new binding specificities on both male and female germ cells. These binding sites can be exploited 1) by direct peptide binding, which in some cases is toxic in itself or 2) by coupling the specific peptide to a cytotoxic small molecule which specifically kills the target cells."

This work has been funded on contract by the CSIRO Food Futures Flagship program with the funding to cease in September 2013 culminating with the final elements of the mouse model proof of principle studies referred to above. A final report to the contractor (CSIRO) is due by February 2014. Dr Michael Holland is the CSIRO contact person and Flagship Chair.

University of Newcastle will retain the Intellectual Property but is prepared to give exclusive licence(s) to exploit that IP. They have expressed their interest in laboratory collaboration in large animal application studies, which would however need to be located at a suitable veterinary faculty/facility.

It should be noted that this large group of scientists & staff (n=150) is uniquely focused on basic contraception research and has good facilities and a collaborative commercial mindset. Other human reproduction academic expertise in Australia (Aitken pers. comm.) is located at Monash University (focus is testicular physiology- resolving male infertility) and Adelaide University (focus is oocyte maturation in vivo and in vitro for IVF ie a pro-fertility focus). These groups are not therefore aligned practically with current MLA/industry R&D priorities related to this project.

Recommendations

MLA should seek to negotiate a confidential non-disclosure agreement with CSIRO at Chief of Division level for specified individual(s) to access and read this basic strategic science report when available.

The industry specification for contraceptive tools/outcomes formulated in this project methodology should be utilised to evaluate likely potential future development and commercialisation outcomes in ruminant livestock. The likely continued presence of female and particularly male steroid hormone production with this germ cell targeted depletion approach is pertinent. The induction phase duration/number of treatments may also be critical from the industry perspective.

If judged appropriate MLA should seek discussions with Chief of Division CSIRO, relevant staff and the report's authors on the likely or potential applicability of the science in their report (or elsewhere as informed by this project) to the cattle and/or sheep and goat industries.

Upon satisfactory agreement a project or program to investigate livestock applications should be determined and designed.

If technically indicated individual or possible joint role(s) of each organisation in any indicated priority application studies for cattle and/or sheep and goats should be negotiated and contracted

as appropriate. Work should be directed at both male and female germ cell targets but with, if necessary, priority on the male.

Other technologies targeting the female

Channel webbing
MLA has recently contracted a project to assess chemical sterilisation by ovarian injection (see section on current MLA projects). In this reviewers critique of this project reference was made to a possibly simpler alternative of channel webbing.

This surgical intervention is conducted manually via the cervix similar to WDOT but aims to transect only the fallopian tube and immediately adjacent attached ligaments to thereby disassociate the severed tubal ends and render the fallopian tube non-functional. Tubal ligation or clamping may be alternatives as in the human patient via key hole surgical technique.

The procedure is currently conducted by Dr Letchford and at least one lay operator known to him. Less mortality is achieved compared with WDOT as there is no interference with the ovarian pedicle and haemorrhage from the ovarian artery is thereby eliminated.

Current difficulties with channel webbing lie with more onerous manipulative technique, no available fit for purpose instruments and the fact that it is NOT currently taught to under-graduate veterinarians. Dr Peter Letchford believes it is warranted to address these issues to provide a simpler, safer and more immediate replacement for WDOT and flank spaying approaches. He has commenced “inventing” preferred instrumentation in his practice.

Recommendation
That MLA investigate and then fund a practical applied research project led by Dr Letchford and with lay assistance to develop a total delivery package for channel webbing. This should include improved instrumentation, possible analgesia, revised and documented preferred manipulative technique for cows and heifers and a plan for under-graduate and post-graduate education and training on an on-going basis. This should preferably be achieved at all current veterinary schools.

This program would seek to provide a practical 5-10 year bridging strategy between current spaying usage (approx. 500,000 animals per year – source MLA report - Survey of Husbandry Practices 2009) and the advent of cost-effective non-surgical on-farm replacement products.

In the event of this latter goal proving elusive however, the judgement by this reviewer is that channel webbing could prove a significant low cost welfare advance able to protect industry from elements of criticism, such as total inaction on spaying, in the interim.

If combined by industry with increased use of the parallel industry strategy of marketing entire males by 2-2.5 years of age (see current project reviews section and recommendations), a dual plan for moderating animal welfare criticism could emerge. This would provide female and male interim measures able to be readily implemented, whilst full replacement technologies are brought to the market-place as envisaged by this review and report.

Peripheral Male Reproductive Targets for Immuno-contraception.

R.A.Bowen 2008 in his review “Male contraceptive technology for non-human male mammals” focuses on the then current state of male contraception and observes in so doing that in general “considerably less effort has been expended than for females”. This remains true across the mammalian species (human and non-human). In addition contraceptive vaccines have a typically quite variable duration of action, are often not highly efficacious and provide only short term

contraception in males. None of these contraceptive vaccine approaches has shown a marketable and clear path toward further development/commercialisation.

However Bowen surmises that “GnRH vaccines may be an exception to this generality” This indeed now appears to be the case with Improvac® from Zoetis Inc. for use in pigs being a commercial example and success (Oonk et al 1998, Brunius et al 2011) but not without significant industry welfare and market drivers ie boar taint and sucker castration.

In contrast Mruk D.D. 2008 – see Table 1 p59, reviewing “New perspectives in non-hormonal male (human) contraception” cites numerous drugs and drugable targets but only two immune-contraceptive sperm studies ie, eppin and semenogelin. This arises because human contraception science properly discounts anti-GnRH vaccine use because of the testosterone suppression effects and therefore seeks more discrete, infertility focused, peripheral reproductive targets eg sperm, seminal fluid, epididymis etc

More recently McLaughlin and Aitken 2011 (Sections 2.5 to 2.7 pp 82-83) reviewed the male target antigen field citing many potential fertility regulating antigens eg izumo, Catsperm 1-4, SP56, eppin etc but conclude that no single antigen yet fufills all the criteria for a human vaccine.

This led them to look at new strategies eg phage display technology, for uncovering novel antigens with high efficacy or a multi-antigen approach or a combination of both. See recommendations under “Other peripheral female reproductive targets” which are equally applicable to the male reproductive system in principle.

In addition Chinese researchers in addressing male contraception have focused on the follicle-stimulating hormone receptor (FSHR) expressed exclusively on the membranes of the Sertoli cells of the testis. Li-Hua Yang et al (2011) showed that immunisation with a synthetic part-peptide from FSHR protein was capable of inducing infertility in male mice, without pathologically damaging the testis or depressing testosterone levels, thus getting closer to a potentially safe human contraceptive vaccine.

However, overall the male contraceptive field is less well researched in general (humans and animals) and much more dependent on these recent non-hormonal approaches. This is especially true in humans, with unfortunately the current ultimate “responsibility” still transferred to women as indicated crudely by the continued high levels of human abortion worldwide (46 million annually quoted by McLaughlin and Aitken 2011 p78).

In contrast, the business of cattle reproduction requires high, efficient and annual female fertility plus the best bull control possible, ie genetically preferred bulls only joined. All other males must be either, controlled and removed from the herd, surgically castrated (as best currently practised)) or ideally rendered totally infertile (sterile) as soon as possible in life but with intact hormonal growth and behaviours to maximise growth efficiency (one future non-surgical scenario). See current MLA project B.NBP.0486.

This male bovine contraceptive ideal is globally sought and is now advancing commercially for cattle, specifically using GnRH immune-contraception. Recent publications on Bopriva® (Zoetis, Parkville Australia) indicate good meat quality and weight gain outcomes in Bos indicus 2-3 year old, destined-for-slaughter bulls compared with surgically castrated controls. Some 188 days (27 weeks) duration of interpreted sub-normal testosterone levels from 91 days (13 weeks) dosing interval was reported in approximately 65% of animals at pasture in Brazil (Amatayakul-Chantler 2013) contrasted with at least 105 days (13 weeks) with the same vaccine using a 49 days (7 week) dosing interval in feedlot cattle in Mexico (Amatayakul-Chantler 2012).

Further progress may be contemplated with continued commercial investment and a focus on desirable industry specifications vis a vis product dose response delivery targets. A long acting, single dose and high efficacy GnRH immunocontraceptive in male cattle however awaits future developments. Efficacy is only one of these three challenges but wide variability is a common

theme in GnRH vaccine publications (see cited sub-review by Kirkpatrick et al 2011 Table 1 p46 in Contraceptive Vaccines for Wildlife: A Review pp40-50)

Recommendations

MLA should actively pursue multiple options/approaches in pursuit of future flexible multi-stringed industry outcomes :

The anti-GnRH vaccine approach (as recommended in the GnRH based immune-contraception section above) for both sexes but particularly males, where for bulls this remains the single most likely to succeed and most commercially advanced technology

The novel exploration of basic kisspeptin biology and control manipulations in cattle and sheep (as recommended for this topic –see section above) which may augment, improve or replace GnRH technology in the medium term if proven to be more biologically efficient ie, when used alone or in combination with an anti-GnRH vaccine.

New application studies targeting germ cell depletion, which is currently being researched by the University of Newcastle here in Australia when applied to males – see recommendations above under “Other peripheral female reproduction targets”. Applications specific to bulls and rams need to be investigated and as a priority over female applications, due to the more pressing need to replace castration.

It should also be clearly noted, and perhaps agreed in principle by industry in advance of any funding, that this latter approach is most likely to lead to the outcome of infertile males but with full male behaviours due to intact testosterone function. A scientific and industry risk assessment in this area should be pursued prior to R&D start up. The adoption of slaughter of male phenotype animals remains an industry option (as per B.NBP.0486 finishing project) which is also a proven productivity win for industry.

This contrasts with upstream level central strategies eg GnRH and Kisspeptin vaccines above, which would deliver an early or traditional castrate/steer outcome but with no inherent productivity gains. This alone would suggest a strategy leaning towards the germ cell depletion approach. Moreover Kisspeptin R&D in the farm animal contraception field is not yet initiated in Australia (or? Globally) and a long science delivery pipeline (? >10+ years) is likely.

Other Technologies/Approaches Targeting the Male

Young Entire Male Marketing

Project B.NBP.0486 was reviewed as a current MLA project relevant to this project topic ie alternatives to castration - See discussion above in current projects section of this report. The marketing of young entire males would directly alleviate the need to castrate any individual so finished, be it via a feedlot or even at pasture.

Recommendations

MLA should carefully appraise the report for all elements of this marketing strategy which constitute “success” factors eg improved profit and productivity, reduced labour, better welfare etc and which together make a compelling case for industry adoption and uptake over time.

MLA should address and resolve where possible all negative factors which might prevent adoption eg adjustments to MSA grading criteria. Any management and bull behavioural concerns of industry could well be actively addressed with concurrent use of GnRH vaccine technology, ie Bopriva® by Zoetis P/Ltd., already in use for management of dairy bull finishing at pasture in New Zealand.

MLA should negotiate with AMPC or other processor representative bodies or processors servicing the northern industry to smooth the way for value based marketing and therefore for future growth in slaughtering of entire males at appropriate grid specification values.

MLA should actively advocate and promote this avenue of production and marketing as an industry win-win for productivity, profit and alternative improved welfare. This would particularly allow early adoption on farm of management which thereby would reduce the total number of castrations conducted.

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