The use of GnRH agonists to suppress fertility in female cattle

1.0 Summary

A significant proportion of beef cattle production in Australia occurs in environments and conditions where it is often not possible to achieve a total segregation of different groups of cattle, both within and between cattle stations. This commonly results in the indiscriminate dispersal of bulls and the occurrence of unwanted pregnancies in cattle that are surplus to breeding requirements. The two classes of female cattle that are typically categorised as surplus to breeding, and are deemed cull females, are young heifers not selected to enter breeding herds and older cows that show reduced reproductive performance. It is usually necessary to retain these heifers and cows for a period of time in order to achieve a weight and body condition that is optimal for sale, and it is during this period that unwanted pregnancies can occur. To prevent pregnancy in cull female cattle the ovaries have been traditionally removed by surgical procedures (spaying). Changes in both industry and community expectations of management practices in livestock production have led to the search for non-surgical alternatives to spaying.

Alternatives to spaying which have been investigated in cattle include immunisation against gonadotrophin releasing hormone (GnRH), GnRH agonist implants, cytotoxic compounds targeted to gonadotrope cells, intra-uterine devices, and the administration of natural and synthetic gonadal steroids. None of the technologies tested to date have shown the profile of efficacy and practicality, and cost, required for a commercial alternative to spaying in female cattle.

GnRH agonists have potential as a technology to achieve long-term contraception in female cattle. Dose-response studies have shown that ovarian activity can be suppressed for greater than 12 months by a single treatment with an appropriate dose of GnRH agonist in a controlled release subcutaneous implant. A commercial, long-term GnRH agonist implant will need to have features that include (1) tissue-inert material, (2) controlled release for at least 12 months and preferably 24 months, (3) release profile with zero order kinetics, (4) ease of application, (5) cost that will be borne by the cattle industry, (6) regulatory acceptance, and (7) other features that make it the contraceptive technology of choice in cattle leading to rapid uptake and displacement of conventional spaying.

GnRH agonists also have potential for controlled, reversible suppression of fertility in female cattle. One application would be to delay puberty in heifers in order to prevent precocious puberty and early pregnancies in extensively-managed herds. A second application would be to suppress fertility for defined periods in heifers and cows for various management objectives; for example, preventing unwanted pregnancies during periods of extended drought. It has been shown that young heifers implanted with GnRH agonist and maintained with bulls do not attain puberty and do not conceive until removal of the GnRH agonist implant.

Further examination of GnRH agonists for fertility management in heifers and cows is warranted given the potential application for both (1) longer-term suppression of fertility to replace spaying and (2) controlled, reversible suppression of fertility. Other contraceptive technologies under investigation do not have this dual application.

It has been estimated that 400,000 - 500,000 heifers and cows combined are spayed annually in northern Australia.

2.0 Introduction

2.1 Cattle production in extensive environments

A large proportion of extensive cattle production in Australia occurs across Northern Australia which is generally regarded as the region north of the Tropic of Capricorn. Around 85% of the landmass of northern Australia is utilised for extensive beef cattle production. Cattle stations owned by families typically have 2,000 - 8,000 cattle whilst pastoral companies usually manage integrated enterprises with between 100,000 - 500,000 cattle.

The total number of cattle across northern Australia averages around 8 million and varies depending on climatic conditions and marketing opportunities. Northern Australia receives seasonally monsoonal rain in summer with considerably less rain in winter and spring. Because of the highly seasonal pattern of rainfall, and the relatively dry and harsh conditions in winter, the mustering of cattle in the more extensive herds for management purposes (e.g. weaning, selection, culling, marketing) commonly occurs twice annually in late-spring and late-autumn.

The bulk of the more extensive beef industry in northern Australia continues to practice year-round mating in which bulls remain within breeding herds continuously. Given the extensive environment and nature of the infrastructure it is often difficult to keep different classes of cattle (e.g. breeding and culled females) as separate herds. It can also be difficult to tightly control the movement of bulls, both within and between cattle stations.

2.2 The need to suppress oestrus in female cattle in extensive environments

The annual replacement of female cattle from breeding herds ranges from 5-10%. Cows in breeding herds that are identified for culling are often in relatively poor condition since they are likely to have recently weaned a calf, or indeed have a calf-at-foot when the decision to cull is made. It is therefore often necessary to retain these cows for a period of between 6-18 months, depending on pasture conditions and market opportunities, to improve their live weight and body condition to optimise their value at sale.

It is highly undesirable for cull cows to become pregnant during the period before sale. This can reduce the value of cows and there are also ethical and welfare implications if cows are pregnant at slaughter. Given the difficulty in controlling the dispersal of bulls in extensive environments, cull cows have traditionally been spayed.

Spaying can result in a transient reduction in growth performance, morbidity or mortality. The mortality rate from spaying has been reduced in the past 5-10 years with the introduction and widespread adoption of the Willis Dropped-Ovary Technique (Jubb et al, 2003). Nonetheless, mortality rate remains at around 1-3% and can be greater if there are a particular set of circumstances including an inexperienced operator.

Another group of female cattle that are often spayed are heifers not selected for breeding. The selection usually occurs at around 20-26 months of age when heifers can vary in live weight from 280-350 kg, depending on genotype, management and pasture conditions. There is an immediate market for some of these heifers but the majority are

retained for between 12-18 months in order to optimise their sale value. These heifers are spayed to prevent pregnancies during the period when they are being prepared for sale.

Hence, there are two main categories of female cattle that are spayed to prevent pregnancies, older cows that have reduced reproductive performance and heifers which are surplus to breeding (Table 1).

An important issue in regard to a non-surgical technology to replace spaying is that the number of cows and heifers treated with the technology would likely be greater than the numbers currently spayed, given both the industry and community view of spaying. Also, producers would be more likely to use a technology that they could apply themselves rather than the need to plan and coordinate for cattle to be spayed. The issues of morbidity and mortality associated with spaying are also highly relevant.

Table 1. Estimates of the number of heifers and cows spayed annually in northern Australia based on historical information.

	Estimated number of animals spayed	
Spaying technique	**Heifers**	**Cows**
Willis Dropped-Ovary	180,000	55,000
Flank	80,000	30,000
Trans-vaginal	40,000	15,000
Total	300,000	100,000

A technology to replace spaying in northern Australia would ideally be effective for at least 12 months and preferably 24 months. The latter would allow female cattle to be retained for a longer period if seasonal conditions did not support growth and body condition.

A further consideration with a technology that replaced spaying is that it would provide management options for sectors of the beef industry that currently does not use spaying, including the industry in southern Australia, although this might be difficult to quantify.

If the technology was reversible there would be important applications to (1) prevent early pregnancies in heifers that show precocious puberty and (2) transiently suppress fertility in breeding herds in response to seasonal conditions or other management considerations.

The prevention of early pregnancies in heifers in extensive environments has several important considerations:

1. Precocious puberty and pregnancy in heifers can result in lifetime negative outcomes if heifers calve at an inappropriate live weight and body condition;
2. Heifers that conceive early may do so out of synchrony with annual cycles of rainfall and pasture availability; this can severely compromise the survival of heifers at calving, and also the survival of calves; and
3. Heifers that conceive early and out-of-season can remain out-of-synchrony with breeding herds which imposes additional management of calves.

The application of a GnRH agonist (see Section 3) to prevent early pregnancies in heifers was demonstrated in an extensive subtropical environment. Zebu crossbred heifers at 14 months of age and around 200 kg received a GnRH agonist implant placed subcutaneous in the ear and they were then maintained together with bulls. During the period with bulls, about 40% of control heifers that had not been implanted became pregnant whereas 5% of heifers implanted with a medium dose of GnRH agonist conceived (Figure 1). At the completion of the first phase of the study, the GnRH agonist implants were removed and previously implanted heifers were placed within breeding herds at the start of a normal breeding season. Heifers that had been treated with GnRH agonist showed the same cumulative pregnancies as heifers that had not been treated (Figure 2). The latter demonstrated the controlled, reversible contraceptive feature of GnRH agonists. The restoration of normal ovarian after the discontinuation of agonist treatment was also demonstrated in cows (D'Occhio et al, 1996).

The increasing adoption of crossbreeding in northern Australia with genotypes that have an earlier age at puberty than Zebu cattle has placed a greater need on controlled, reversible contraceptive technology to prevent precocious puberty and early pregnancies in heifers. There is also an emerging opportunity for young cull heifers from northern herds to be relocated to feedlots and it is important to prevent pregnancies in these heifers. Similarly it is critical to prevent pregnancies in heifers that enter the live export market.

The need for new technology to manage reproduction in female cattle in extensive beef herds is therefore being driven by the requirement to replace conventional spaying and also by other changes in the industry.

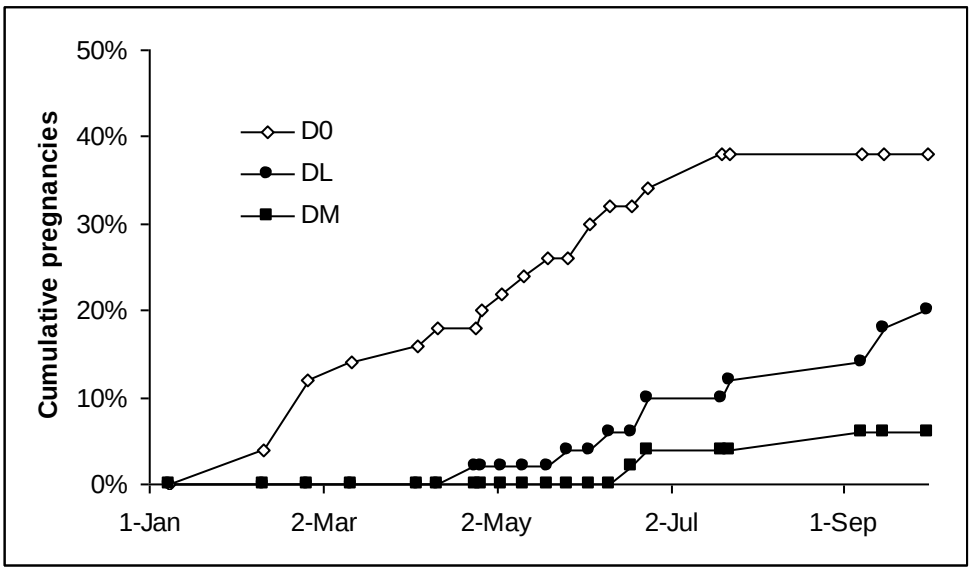

Figure 1. Cumulative pregnancies in Zebu crossbred heifers treated with a low-dose (DL) or medium-dose (DM) GnRH agonist implant comprising deslorelin, and control heifers (DO) not treated. For details see text (D'Occhio MJ and Fordyce G, unpublished).

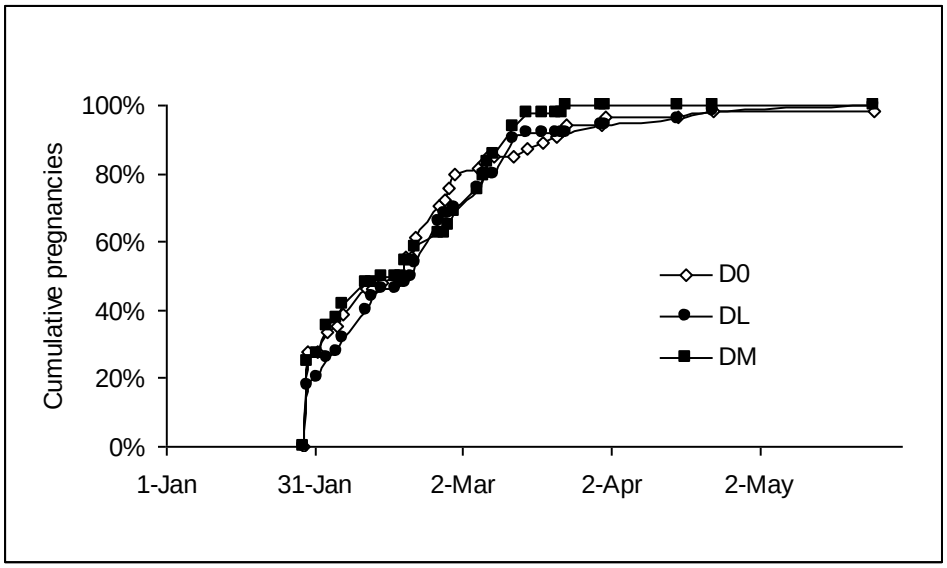

Figure 2. Cumulative pregnancies in Zebu crossbred heifers previously treated with a low-dose (DL) or medium-dose (DM) GnRH agonist implant comprising deslorelin, and control heifers (DO) not treated. GnRH agonist implants were removed 4 months before heifers were introduced into breeding herds (31 Jan). For details see text (D'Occhio MJ and Fordyce G, unpublished).

2.3 Control of reproduction in female cattle in annual management cycles

As noted earlier, northern Australia receives seasonally monsoonal rainfall that typically occurs during summer. The winter period is relatively dry and harsh and cattle are not usually handled during this time. In most management systems, therefore, cattle are mustered and handled in late-spring (before the wet season) and late-autumn (after the wet season and before the dry season).

Although for most enterprises in northern Australia bulls are continuously present in breeding herds, the majority of pregnancies tend to occur during mid- to late-summer (wet season) given the role of nutrition in reproductive function. Calving usually occurs from late-spring to early-summer.

The control of fertility in female cattle in northern herds needs to be synchronised with the above cycles of management and reproduction. Contraceptive technology is likely to be applied as follows (Muster 1, late-spring; Muster 2, late-autumn):

1. Cows in breeding herds can be identified for culling at Muster 1 or Muster 2; a contraceptive technology can be applied at either time and the decision on sale made at the muster 12 or 18 months later;

2. Prepubertal and pubertal heifers (12-16 months of age) that are identified for culling at Muster 1 can be treated with a contraceptive technology and a decision on sale made at the muster 12 or 18 months later;
3. Non-pregnant postpubertal heifers (20-24 months of age) that are identified for culling at Muster 1 can be treated with a contraceptive technology and a decision on sale made at the muster 12 or 18 months later;
4. Prepubertal and pubertal heifers (12-16 months of age) that are deemed suitable for breeding in Muster 1 can be treated with a controlled, reversible contraceptive technology that will prevent pregnancies for around 10-12 months until the following breeding season; and
5. Cows in breeding herds that are in relatively poor condition, and non-pregnant at Muster 2 due to unfavourable conditions, can be treated with a controlled, reversible contraceptive technology to prevent pregnancies from occurring during the ensuing dry winter, and for fertility to return in the following breeding season.

2.4 The choice of contraceptive technology

The contraceptive technology of choice is guided by the anti-fertility objective (D'Occhio, 1993; Kutzler and Wood, 2006). If the requirement is to prevent conception whilst retaining reproductive and associated behaviours, then the target for a contraceptive technology can be the gametes, fertilisation process, and zygote/embryo. If, however, the requirement is to suppress mating behaviour and prevent pregnancy then it is necessary to target the reproductive axis at the hormones that stimulate behaviour. Arguably the most effective way to suppress the reproductive endocrine system is to block the action of gonadotrophin releasing hormone (GnRH). GnRH is a neuropeptide that is released from the base of the brain and which stimulates the reproductive hormone cascade that leads to the synthesis and secretion of gonadal steroids from the ovaries and testes. Gonadal steroids are responsible for reproductive and aggressive bahaviours.

In the absence of next-generation contraceptive technology that can be applied to manage fertility in female cattle, the beef industry continues to use surgical procedures.

As noted above, the surgical approaches used in female cattle are flank spaying, trans-vaginal spaying and more recently the Willis Dropped-Ovary Technique. All three procedures can be used in heifers and cows but the typical use is as follows:

1. Flank spaying - relatively young heifers unsuitable for trans-vaginal spaying and cows;
2. Trans-vaginal spaying - heifers and cows; and
3. Willis Dropped-Ovary - heifers and cows.

The Willis Dropped-Ovary Technique is generally regarded as a major advance on flank and trans-vaginal spaying in terms of (1) reduced stress, (2) reduced morbidity and (3) reduced mortality (Jubb et al, 2003). This technique has become the industry standard.

However, given that all three of the above procedures involve surgical intervention, both industry and the general public are seeking to find an acceptable non-surgical approach to prevent unwanted pregnancies in heifers and cows in extensive environments. The beef industry wishes to show leadership in adopting a technology to replace spaying and Meat and Livestock Australia is actively exploring alternatives.

An intra-uterine device (Figure 3) was evaluated in Zebu cattle in northern Australia but proved unsatisfactory (Fordyce et al, 2001) despite a report that claimed the device prevented pregnancy and also improved growth performance in cattle (Turin et al, 1997). Patents exist for the contraceptive (No. 5205055) and growth promoting (EP0787427) features of the device.

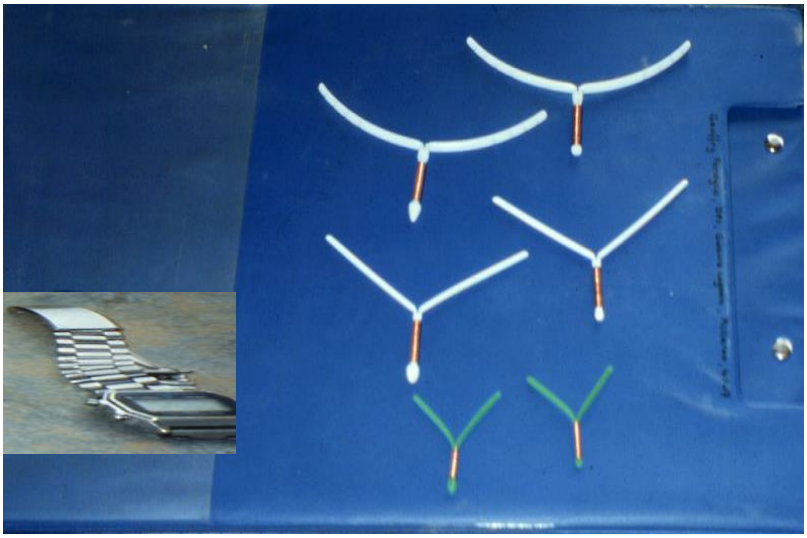

Figure 3. Intra-uterine devices designed for use in cattle. A device is placed in each of the uterine horns: lower device, relatively small heifers; middle device, medium size heifers and cows; upper device, larger cows.

3.0 Use of GnRH agonists to suppress fertility in female cattle

3.1 Ovarian physiology in female cattle

Female cattle are polyoestrus and continue to show regular ovarian cycles until pregnancy occurs. Reproductive activity in female cattle is reliant on a functional reproductive endocrine system that involves the hypothalamus, anterior pituitary gland and

gonads (hypothalamic-pituitary-gonadal axis) (Figure 4). Gonadotrophin releasing hormone (GnRH) is released from the base of the brain (hypothalamus) and is transported by the hypothalamo-hypophyseal portal blood system to the anterior pituitary gland where it stimulates the synthesis and secretion of the two gonadotrophic hormones, luteinising hormone (LH) and follicle stimulating hormone (FSH) (Figure 5). The gonadotrophic hormones in turn are transported by the systemic circulation to the ovaries where they induce steroidogenesis and gametogenesis.

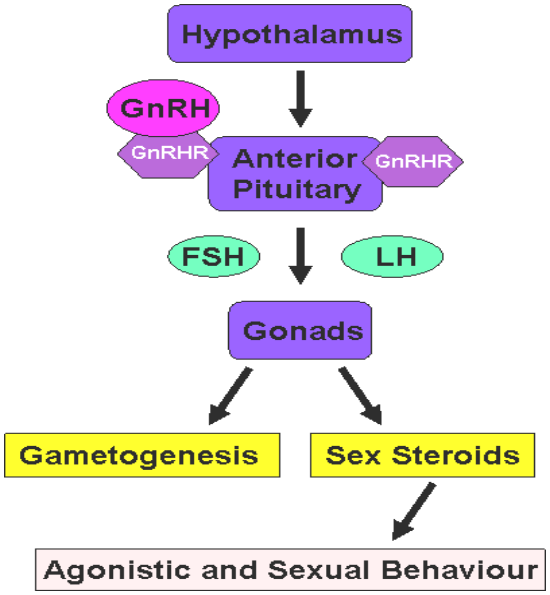

Figure 4. The hypothalamic-pituitary-ovarian axis in female cattle. See text for details.

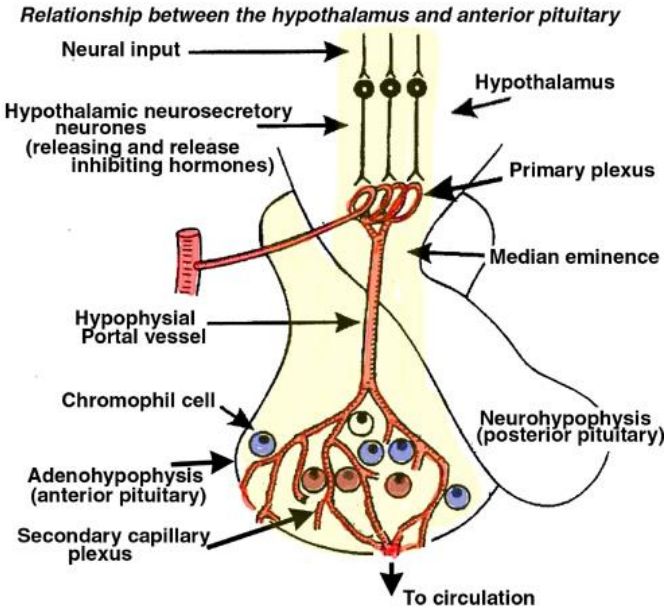

Figure 5. Schematic representation of the hypothalamo-hypophyseal blood capillary portal system that transports neuropeptides released at the base of the brain to the anterior pituitary gland. GnRH is amongst the neuropeptides.

Ovarian follicular growth in cattle occurs in 'waves' (Figure 6). In each follicular wave, a cohort of 6-10 follicles is stimulated to grow in response to a transient increase in the concentrations of FSH in circulation (Figure 7). A dominant follicle emerges from the cohort and continues to grow towards ovulation whilst the remaining follicles undergo regression (Figure 6, Figure 7). The dominant follicle cannot complete maturation and ovulate if a corpus luteum is present that secretes progesterone (Figure 6, Figure 7). Concentrations of progesterone in circulation during the luteal phase of the oestrous cycle suppress the frequency of pulsatile secretion of GnRH, which in turn leads to LH secretion that is insufficient to support the final development and maturation of dominant follicles. Regression of the corpus luteum and decline in circulating concentrations of progesterone results in increased secretion of LH which promotes final maturation of the dominant follicle, and the pre-ovulatory surge release of LH stimulates ovulation (Figure 6, Figure 7).

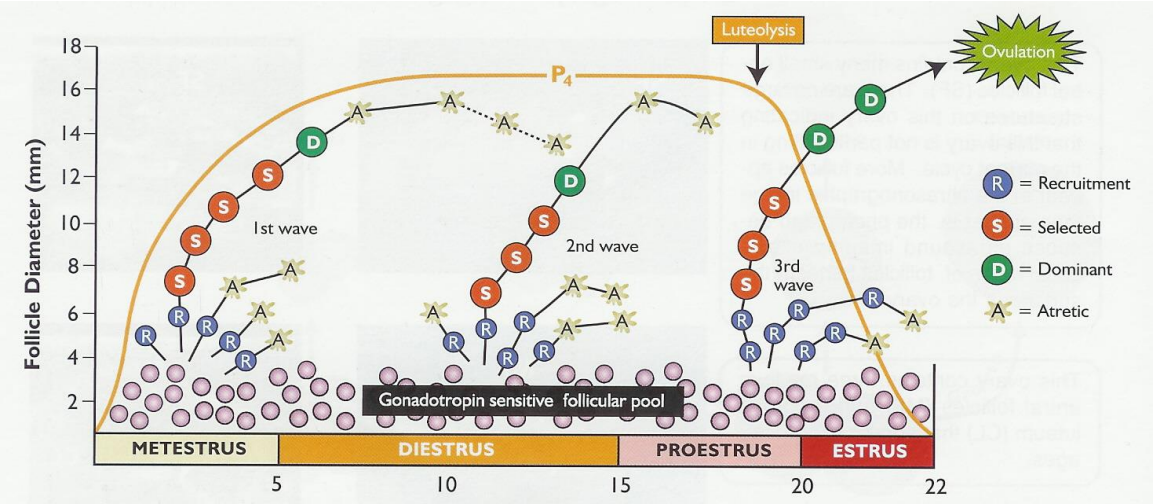

Figure 6. Schematic representation of ovarian follicular waves in female cattle. See text for details.

3.2 Different forms of GnRH

GnRH occurs in at least two forms in mammals, Type 1 GnRH and Type 2 GnRH (Pawson et al, 2003; Metallinou et al, 2007). Type 2 GnRH is expressed in the gonads and other somatic tissues where it is thought to have local autocrine and paracrine actions (Metallinou et al, 2007). GnRH receptors are also found in somatic tissues (Aguilar-Rojas and Huerta-Reyes, 2009).

3.3 Association of GnRH and GnRH receptors with somatic tumours

GnRH peptide and GnRH receptors have been described in malignant tumours in a range of somatic tissues (Limonta et al, 2003; Marelli et al, 2006; Aguilar-Rojas and Huerta-Reyes,

2009). The presence of GnRH receptors in tumours provides the potential for clinical management of some tumours directly with analogues of GnRH (Sundaram et al, 2009).

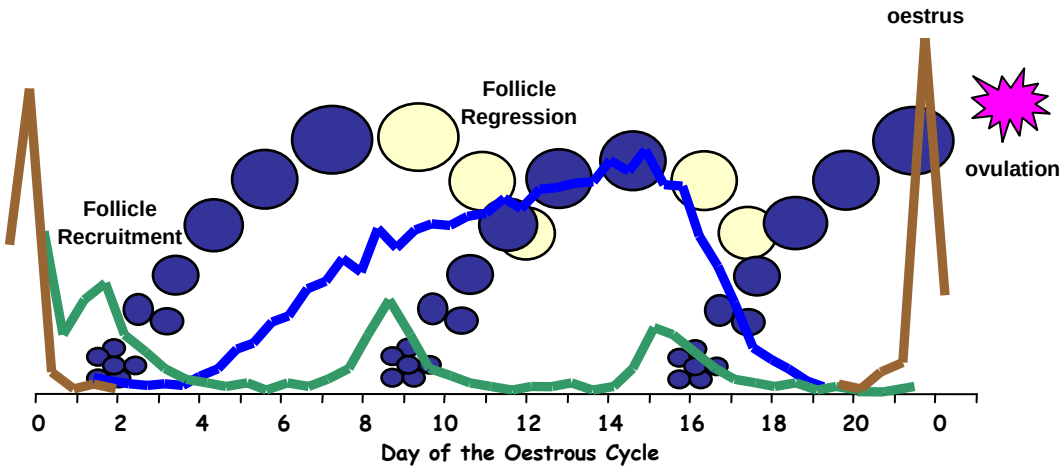

Figure 7. The interrelationships between hormones and ovarian follicular waves in female cattle. Successive follicular waves (blue circles) are initiated by transient increases in the concentrations of FSH (green line) in circulation. The dominant follicle of a follicular wave cannot proceed to ovulation if a corpus luteum is present that secretes progesterone (blue line). Regression of the corpus luteum leads to increased secretion of LH that promotes final growth and maturation of the dominant follicle, and ovulation, which occurs in response to the pre-ovulatory surge release of LH (brown line). See also Figure 6.

3.4 *Agonists of gonadotrophin releasing hormone (GnRH)*

As noted above, GnRH is released from the hypothalamus and binds to specific GnRH receptors on gonadotrope cells in the anterior pituitary to stimulate the synthesis and secretion of LH and FSH (Figure 4). The binding of GnRH to receptors causes the receptors to dimerise (receptor microaggregation; Janovick and Conn 1996) and this is followed by the internalisation of GnRH-GnRH receptor complexes within the cytoplasm of gonadotrope cells. This leads to a temporary depletion of GnRH receptors on the surface of gonadotrope cells, and the cells are transiently unresponsive to further stimulation from GnRH (D'Occhio et al, 2000). Receptors are replenished at the surface of gonadotrope cells over 1-2 hours after stimulation by GnRH and the response of the cells to GnRH is restored (D'Occhio et al, 2000).

GnRH is a 10 amino acid neuropeptide (Table 3) and is rapidly metabolised after release into the general circulation (Table 4). This restricts the use of natural sequence GnRH in therapeutic applications.

Table 3. Amino acid sequence of natural GnRH and GnRH agonists. A key feature of GnRH agonists is the substitution of Gly at amino acid position 6 with a D-amino acid. This substitution slows proteolytic cleavage at amino acid position 6 and extends the half-life of GnRH agonists in circulation (Table 4). A second feature of some GnRH agonists is the replacement of Gly at amino acid position 10 with NHEt. This second feature increases the affinity of GnRH agonists for GnRH receptors on gonadotrope cells in the anterior pituitary gland. The two features of GnRH agonists, combined, increases the biological potency of agonists compared with natural sequence GnRH.

	Amino acid number									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Decapeptides										
GnRH	pGlu	His	Trp	Ser	Tyr	Gly	Leu	Arg	Pro	Gly NH_2
Nafarelin	pGlu	His	Trp	Ser	Tyr	D-Nal	Leu	Arg	Pro	Gly NH_2
Triptorelin	pGlu	His	Trp	Ser	Tyr	D-Trp	Leu	Arg	Pro	Gly NH_2
Nanopeptides										
Buserelin	pGlu	His	Trp	Ser	Tyr	D-Ser(tBu)	Leu	Arg	Pro	NHEt
Deslorelin	pGlu	His	Trp	Ser	Tyr	D-Trp	Leu	Arg	Pro	NHEt
Goserelin	pGlu	His	Trp	Ser	Tyr	D-Ser(tBu)	Leu	Arg	Pro	Azagly-NH_2
Histerelin	pGlu	His	Trp	Ser	Tyr	D-His(Bzl)	Leu	Arg	Pro	NHEt
Leuprolide	pGlu	His	Trp	Ser	Tyr	D-Leu	Leu	Arg	Pro	NHEt

Given the rapid metabolism of GnRH and relatively short half-life in circulation, agonists of GnRH were developed (Table 3) that have three distinguishing features compared with natural sequence GnRH:

1. Significantly reduced metabolism and longer half-life in circulation;
2. Greater affinity for the GnRH receptor on gonadotrope cells; and
3. Greater biological potency.

Table 4. Half-life in circulation and relative biological potency of natural sequence GnRH and GnRH agonists.

	Half-life in circulation	**Relative Potency**
GnRH	5-10 min	1
Buserelin	80 min	20-40
Deslorelin	70-80 min	120-150
Goserelin	4.5 h	50-100
Histerelin	< 60 min	100
Leuprolide	90 min	50-80
Nafarelin	3-4 h	200
Triptorelin	3-4 h	36-144

Deslorelin is a widely used GnRH agonist with a relatively high biological potency (Table 4). Deslorelin differs from natural sequence GnRH in two fundamental ways:

1. Gly at amino acid position 6 is replaced with D-Trp: position 6 is the primary enzymatic cleavage point of GnRH and replacement with D-Trp significantly reduces the rate of metabolism; and
2. Gly at amino acid position 10 is removed: the removal of Gly^{10} results in greater affinity for the GnRH receptor on gonadotrope cells.

3.5 The response of gonadotrope cells to GnRH agonists

GnRH agonists were initially developed for use as replacement for GnRH in reproductive dysfunctions that resulted from a lack of endogenous GnRH. It was subsequently found that whilst the initial response to continued treatment with agonist involved the release of LH and FSH, this was followed by a suppression of pulsatile secretion of both gonadotrophins (D'Occhio et al, 1999, 2000).

The acute-phase response to treatment with GnRH agonist involves typical binding of agonist to specific GnRH receptors on gonadotrope cells, receptor microaggregation, internalisation of agonist-receptor complexes into the cytoplasm, and stimulation of signal transduction processes that lead to the release and synthesis of LH and FSH (D'Occhio et al, 1999, 2000).

The chronic-phase response to treatment with GnRH agonist is characterised by a lack of GnRH receptors on gonadotrope cells (receptor downregulation) and the disruption of post-receptor binding signal transduction processes (desensitisation) (D'Occhio et al, 2000).

In cattle, the acute-phase occurs during the first 24-36 hours of treatment with GnRH agonist and the chronic-phase response persists provided that agonist is maintained in circulation (D'Occhio et al, 2002).

The acute-phase and chronic-phase responses to continued treatment with GnRH agonist has led to pro-fertility (acute phase) and anti-fertility (chronic phase) applications of GnRH agonists (D'Occhio et al, 1999, 2000).

3.6 Response of females to treatment with GnRH agonist

Females of all mammalian species show the same response to treatment with GnRH agonist. The acute-phase response involves the release of LH and FSH and this has been used to develop GnRH agonist protocols that induce ovulation. Examples include the product Receptal® which comprises the GnRH agonist buserelin and is used as an injectable to induce ovulation in cattle, and Ovuplant® which comprises the GnRH agonist deslorelin and is used as a subcutaneous implant to induce ovulation in horses.

As noted above (Figure 7), the initiation of an ovarian follicular wave in females occurs in response to a transient increase in circulating concentrations of FSH (i.e. pulse of FSH). The chronic-phase response to treatment with GnRH agonist is associated with a lack of pulsatile secretion of FSH and, accordingly, ovarian follicular waves fail to occur in females receiving continuous treatment with agonist (D'Occhio et al, 1999, 2000). Females treated long-term with a GnRH agonist therefore have suppressed ovarian function and fail to ovulate.

It was shown that ovarian activity in heifers and cows could be suppressed for greater than 12 months with a controlled release GnRH agonist implant positioned subcutaneous in the ear (D'Occhio et al, 2002).

In women, GnRH agonist implants reduce concentrations of oestradiol in circulation and have been used in the management of oestrogen-dependent conditions such as endometriosis, uterine fibroids and some breast cancers.

3.7 Response of males to treatment with GnRH agonist

Males of all mammalian species show the same acute-phase response to treatment with GnRH agonist and the release of LH has been used to stimulate testosterone secretion from the testes which provides an index of prevailing steroidogenic capacity (D'Occhio et al, 1997, 1998).

Pulsatile secretion of LH and FSH is suppressed in males during the chronic-phase response to continuous treatment with GnRH agonist. However, males of different species show different testicular responses to a lack of pulsatile secretion of LH. For example, bulls and red deer stags show an increase in testosterone secretion whilst male dogs and men show a decrease in testosterone secretion (D'Occhio et al, 1999, 2000).

Hence, GnRH agonists cannot be applied universally across males of mammalian species to suppress reproductive function. The product Suprelorin® comprises the GnRH agonist deslorelin in a subcutaneous implant that suppresses testicular function long-term in male dogs. A wide range of GnRH agonist implants have been developed for the management of prostatic carcinoma in men.

3.8 Choice of GnRH agonist

There are many agonists available that have the potential to be formulated with controlled release technology and used for reproductive management. There are also many suppliers of the most utilised agonists.

GnRH agonists that are already approved for use in livestock (e.g. deslorelin, triptorelin) would be attractive candidates for other applications in livestock.

Considerations for the use of GnRH agonists in Australia include whether approval to import will be provided by AQIS and also whether approval for use in cattle will be provided by APVMA.

The GnRH agonist deslorelin could be regarded as the most likely agonist to satisfy the product performance requirements for the longer-term suppression of ovarian function in cattle. The precedence for deslorelin is Suprelorin® which suppresses testicular function in dogs from 6 to 12 months. Deslorelin implants have also been shown to suppress ovarian function and prevent pregnancies in heifers and cows for over 12 months (D'Occhio et al, 2002). Triptorelin is a second GnRH agonist with potential for use in cattle and has been evaluated by the APVMA for use in pigs (APVMA June 2016).

The delivery technology (efficacy, cost) is likely to be the limiting factor in the development of GnRH agonist implant for longer-term oestrous suppression in cattle (https://www.mla.com.au/research-and-development/search-rd-reports/final-report-details/Animal-Welfare/Efficacy-of-a-GnRH-agonist-delivered-via-a-slow-release-implant-technology-to-control-reproductive-function-of-the-female-bovine-reproductiv/2926).

3.9 Residues

GnRH agonists are small peptides and are not accumulated as residues in tissue, which occurs with natural and synthetic gonadal steroids. Issues of hormonal residues therefore should not apply to GnRH agonists.

3.10 Safety

GnRH agonist implants that need to be applied subcutaneous with a relatively large needle or trochar could be considered safe and do not pose major OH&S issues.

3.11 Method of administration

Controlled release implants, placed subcutaneous, would appear to be the most effective method to deliver GnRH agonists longer-term. A GnRH agonist bioimplant proved an effective technology to deliver the agonist deslorelin for greater than 12 months in heifers and cows when placed subcutaneous in the ear (D'Occhio et al, 2002).

The composition of implants will be determined by available proprietary technology but the implant material should ideally be inert and compatible with multiple-dispensing technology.

GnRH agonists can be prepared as a liquid injectable but this is method of application is usually restricted to single administration. Receptal® is an example in cattle. Next generation delivery technology such as nanoparticles may have potential.

3.12 Growth in female cattle treated with GnRH agonist

In a long term study over 12 months, there were no consistent differences in changes in live weight between heifers (Table 5) and cows (Table 6) implanted with GnRH agonist and respective contemporary groups of females that were not implanted (D'Occhio et al, 2002). It should be noted that in the latter study cattle were maintained under extensive management where the potential for growth would have been influenced by the availability of pasture. Heifers immunised against GnRH and maintained in a feedlot showed reduced growth performance compared with untreated heifers (Adams and Adams, 1990; Adams et al, 1990; Prendiville et al, 1995; Bell et al, 1997). Also, heifers immunised against GnRH and maintained on relatively good pasture had different carcass attributes compared with contemporary untreated heifers (Jeffry et al, 1997). Given that GnRH immunisation and treatment with GnRH agonist would be presumed to result in similar suppression of ovarian follicular growth and reduced secretion of steroids, in particular oestradiol, it could be predicted that heifers and cows treated with GnRH agonist could show a different growth performance to untreated contemporaries. However, this would only be expected to occur if nutrition was such that differences in growth potential could be expressed (e.g. feedlot, good pasture), and these conditions typically do not occur in the extensive grazing environments of northern Australia and hence the findings in Table 5 and Table 6.

Table 5. Live weight for control heifers and heifers treated with a GnRH agonist implant under extensive grazing in northern Australia. Numbers in parentheses are number of animals (from D'Occhio et al, 2002).

	Month of treatment[a]			
	0	4	8	12
Station A				
Control	307 ± 4 (10)a	309 ± 7 (9)a	316 ± 6 (16)a	398 ± 8 (11)b
GnRH agonist	303 ± 2 (51)a	303 ± 4 (27)a	314 ± 2 (51)b	388 ± 3 (51)c
Station B				
Control	294 ± 8 (8)a,b	270 ± 7 (8)b	307 ± 8 (7)a	360 ± 12 (8)c
GnRH agonist	307 ± 5 (41)a	283 ± 5 (39)b	306 ± 5 (41)a	373 ± 5 (40)c
Station C				
Control	316 ± 9 (10)a	345 ± 11 (10)a,b	368 ± 5 (3)b,c	396 ± 11 (13)c
GnRH agonist	307 ± 4 (39)a	337 ± 3 (39)b	354 ± 5 (35)c	387 ± 6 (39)d

Means within rows without a common letter (a–d)differ ($P < 0.05$).

[a] There were no differences in live weight between control heifers and heifers treated with GnRH agonist.

Table 6. Live weight for control cows and cows treated with a GnRH agonist implant under extensive grazing in northern Australia. Numbers in parentheses are number of animals (from D'Occhio et al, 2002).

	Month of treatment[a]			
	0	4	8	12
Station A				
Control	459 ± 15 (10)a	418 ± 44 (3)a,b	393 ± 15 (12)b	460 ± 20 (10)a
GnRH agonist	445 ± 8 (48)a	415 ± 11 (23)b	414 ± 8 (48)b	471 ± 9 (46)c
Station B				
Control	308 ± 8 (11)a,b	285 ± 6 (11)a	317 ± 7 (9)b	382 ± 15 (10)c
GnRH agonist	317 ± 5 (48)a	298 ± 5 (47)b	330 ± 6 (47)a	388 ± 5 (48)c
Station C				
Control	407 ± 12 (10)a	439 ± 11 (10)a	416 ± 6 (3)a	502 ± 15 (6)b
GnRH agonist	401 ± 8 (37)a	427 ± 7 (37)b	425 ± 7 (37)b	462 ± 8 (37)c

Means within rows without a common letter (a–c) differ ($P < 0.05$).

[a] There were no differences in live weight between control cows and cows treated with GnRH agonist, except for Station C at 12 months; the latter was due to the introduction of a new group of control cows.

3.13 Other technologies to suppress reproductive function

3.13.1 GnRH antagonists

Antagonists of GnRH also interfere with the action of endogenous GnRH at gonadotrope cells in the anterior pituitary but the mechanism of action of antagonists differs to that of GnRH agonists. GnRH antagonists bind to the specific GnRH receptor on gonadotrope cells but receptor internalisation (receptor downregulation) does not occur and the disruption of signal transduction processes (desensitisation) also does not occur. The biology of GnRH antagonists and methods of delivery were recently reviewed (Mezo and Manea, 2009). A GnRH antagonist suppressed testicular function in rams and bulls (Jimenez-Severiano et al, 2007). GnRH antagonists are used extensively in IVF programs to control endogenous sectretion of LH and FSH and this field was reviewed recently (Devroey et al, 2009). The development of GnRH antagonists for use in the management of reproduction in animals, and in particular livestock, is restricted by the greater complexity and associated greater cost of antagonists compared with GnRH agonists.

3.13.2 GnRH vaccines

As noted above, there are several sites within the reproductive axis that can be targeted for an anti-fertility outcome. Active immunisation against GnRH has received considerable attention and a GnRH vaccine, Vaxstrate®, was commercialised for use in heifers and cows (Hoskinson et al, 1990). Vaxstrate® was discontinued as there was relatively large variation in the number of heifers and cows that showed an immunological response necessary to

achieve adequate immuno-neutralisation of endogenous GnRH, and long-term suppression of ovarian function was not consistent.

GnRH vaccines induce an immunological response that results in the production of anti-GnRH antibodies that are present in systemic circulation. The hypothalamo-hypophyseal portal system (Figure 5) is part of the general circulation. Provided that titres of anti-GnRH antibodies are adequate, the antibodies bind GnRH that is released into the median eminence and thereby prevent GnRH from binding to gonadotrope cells in the anterior pituitary to cause the release of LH and FSH (Figure 5). This results in immuno-contraception that is maintained provided that anti-GnRH antibodies are above the threshold to neutralise endogenous GnRH.

GnRH vaccines that are currently available for use in animals include:

1. Equity® - suppresses ovarian function in fillies and mares for 3-6 months;
2. Bopriva™ - suppresses testicular function in bulls for 2-4 months;
3. Improvac® - suppresses testicular function on boars for around 6 months; and
4. GonaCon™ - suppresses gonadal function in wildlife including deer for 2-3 years and also induced long-term contraception in bison.

The above vaccines require a primary and secondary vaccination except for GonaCon™ which in a number of studies induced a sustained immunological response to GnRH following a single vaccination. The single vaccination with GonaCon™ led to considerable interest in the potential use of GonaCon™ in cattle. In a series of studies in cattle by D'Occhio and colleagues, single vaccination with GonaCon™ was not effective in generating significant anti-GnRH antibody titres and it was concluded that single vaccination is only effective in animals that have previously been exposed to environmental *Mycobaterium avium*, a component of the vaccine (https://www.mla.com.au/research-and-development/search-rd-reports/final-report-details/Animal-Welfare/GonaCon-trial-in-heifers/183; https://www.mla.com.au/research-and-development/search-rd-reports/final-report-details/Animal-Welfare/GonaConTM-trial-in-bull-calves/184; https://www.mla.com.au/research-and-development/search-rd-reports/final-report-details/Integrity-Sustainability/GonaConTM-trial-in-bull-calves-2/2932).

Notwithstanding the limitations with practical application of GonaCon™, vaccination with GonaCon™ can induce longer-term suppression of gonadal activity in cattle (D'Occhio et al 2014) (https://www.mla.com.au/research-and-development/search-rd-reports/final-report-details/Integrity-Sustainability/GonaConTM-trial-in-bull-calves-2/2932).

3.13.3 Cytotoxic compounds

A potential approach to permanently suppress reproductive function is to ablate the gonadotrope cells in the anterior pituitary using cytotoxic compounds. This has been demonstrated, in principle, with pokeweed antiviral protein (PAP; Nett et al, 2003). In the latter study, PAP was conjugated to a GnRH agonist and administered to male dogs as a single injection. The agonist delivers the cytotoxic compound to gonadotrope cells where it enters the cell as part of the GnRH receptor internalisation process. The cytotoxic compound causes cell death by blocking protein synthesis or by other mechanisms that result in apoptosis. In a preliminary trial in cattle, treatment with PAP did not suppress testicular function in bulls (D'Occhio MJ and Kenny A, unpublished).

3.13.4 Anabolic steroids

Both natural (Kniffen et al, 1999) and synthetic (Floyd et al, 1989) anabolic steroids have been shown to suppress reproductive function in female cattle but steroids have not been developed or marketed specifically for fertility control. The three classes of gonadal steroids, oestrogens, androgens and progestagens, all suppress reproduction (Heitzman et al, 1979; Moran et al, 1990). It is highly unlikely that anabolic gonadal steroids will be developed as anti-fertility technology in female cattle given the major issues associated with steroid hormone residues in meat.

4.0 Methods to evaluate ovarian function in cattle

Ovarian function in cattle can be evaluated by a number of methods. Manual palpation of the ovaries by an experienced operator can be used to detect large follicles and the presence of a corpus luteum. Concentrations of progesterone in circulation determined at 10-14 day intervals provide information on whether females are undergoing regular oestrous cycles. Trans-rectal ultrasonography provides a very accurate assessment of structures (follicles, corpus luteum) present on the ovary, it is immediate, and does not require the collection and processing of blood samples and hormone analyses. Ultrasonography is therefore the technology of choice for rapid and accurate determination of ovarian status in female cattle.

5.0 Selected literature on GnRH agonists

Selected literature on GnRH agonists in cattle is included in Section 8.

6.0 Selected literature on GnRH immunisation

Selected literature on GnRH immunisation in cattle is included in Section 9.

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