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Gonadotropin-Releasing Hormone (GnRH) Analogs or Active Immunization Against GnRH To Control Fertility in Wildlife

Susan E. Becker and Larry S. Katz

Abstract: The administration of analogs, both agonists and antagonists, of GnRH and immunization against GnRH have been investigated for their ability to control reproductive function in domestic species. These methods can be used to inhibit the secretion of gonadotropins, the necessary stimulants for steroidogenesis and gametogenesis, thereby potentially preventing ovulation and inhibiting spermatogenesis. Induction of infertility in this manner could be used for nonlethal population control of wildlife species. Relatively little research has been done in this area. This chapter reviews relevant studies with domestic species and discusses results from studies with wildlife species.

Keywords: gonadotropin-releasing hormone, GnRH agonist, GnRH antagonist, GnRH immunoneutralization, wildlife contraception

Introduction

Because hunting and natural mortality cannot control wildlife populations everywhere, there is increasing demand for the development of nonlethal methods for population control of both free-roaming and captive wildlife. Therefore, fertility control through administration of contraceptive agents is being investigated. The ideal contraceptive agent should be (1) reversible (for some species), (2) suitable for remote delivery, (3) effective with only a single administration, (4) unable to contaminate the food chain, (5) without harmful side effects, and (6) without effect on social behavior. Although steroid hormone treatments have been used successfully for fertility control in nondomestic animals (see review by Kirkpatrick and Turner [1991]), the possibility exists for steroids to enter the food chain. A nonsteroidal hormone such as gonadotropin-releasing hormone (GnRH), a small peptide, would not pass through the food chain because when ingested it would be cleaved to its constituent amino acids. Relatively little work has been done to investigate the effectiveness of GnRH as a contraceptive agent in nondomestic species.

Gonadotropin-releasing hormone, synthesized in the hypothalamus of both males and females, is a key regulator of reproduction in mammals. Released from the hypothalamus in a pulsatile pattern, it travels via the portal vasculature to the anterior pituitary, where it stimulates release of the gonadotropins, luteinizing hormone (LH), and follicle-stimulating hormone (FSH). These gonadotropins enter the circulation and regulate both steroidogenesis and gamete maturation in the gonads (Conn 1994). More specifically, in the female, FSH stimulates follicular growth and maturation, and LH induces ovulation and corpus luteum formation. In the male, the direct role for FSH in spermatogenesis is uncertain, and LH causes the Leydig cells of the testis to produce testosterone which is necessary for gametogenesis. FSH, in the presence of LH, stimulates estradiol production from both the ovary and the testis. The steroids secreted from the gonads feed back to the hypothalamus and pituitary to regulate GnRH and gonadotropin synthesis and release (see fig. 1).

It is possible to make the pituitary refractory to GnRH by administering GnRH, or an agonist of GnRH, in a continuous manner, rather than in the physiological pattern of pulses. Prolonged, continuous infusion of GnRH, especially at high concentrations, inhibits gonadotropin secretion (Belchetz et al. 1978), and that results in loss of gonadal function. Initially, pituitary desensitization is thought to result from loss of pituitary cell-surface receptors for GnRH by internalization of occupied receptors (Conn and Crowley 1991). Later, as receptor numbers recover due to recycling (Hazum and Conn 1988) and homologous upregulation (Conn et al. 1984, Braden and Conn 1990), desensitization may be maintained because the receptors become dissociated from their second messenger system (Conn and Crowley 1991).

Controlling the amount and pattern of GnRH stimulation to the pituitary affects gonadotropin synthesis and secretion, thereby affording a potential method of controlling fertility in both males and females. Administration of GnRH agonists or antago-

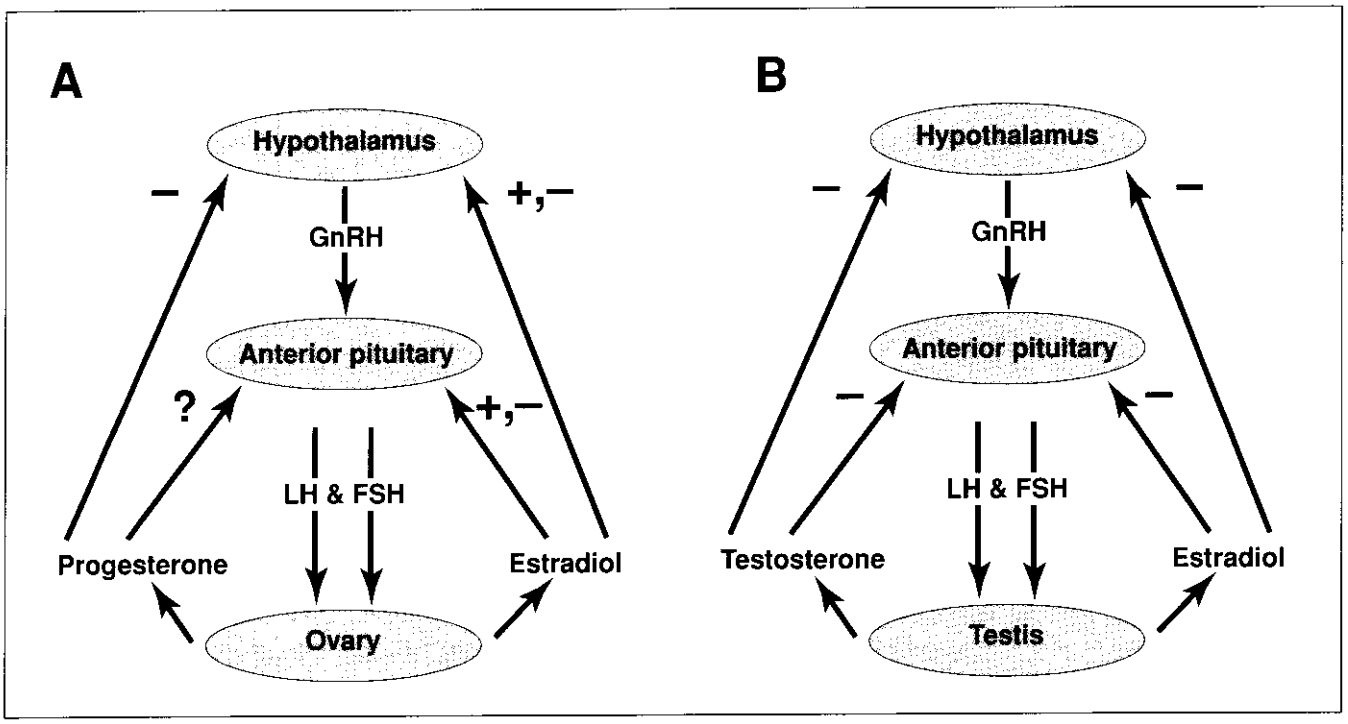

Figure 1. Pathways of positive (+) and negative (–) feedback of gonadal steroids on the hypothalamic–pituitary axis in (A) the female and (B) the male. The effects of estradiol in the female are positive or negative, depending upon the stage of the estrous cycle. Question mark (?) indicates relatively insignificant effects.

nists, as well as immunization against GnRH, have been tested for their ability to suppress reproductive function in humans and domestic animals, yet little work has been done in this area with wildlife.

GnRH and GnRH Agonists

Large doses or chronic administration of GnRH or GnRH agonists can inhibit gonadotropin secretion by pituitary desensitization. Agonists are often preferred, both clinically and experimentally, over GnRH itself due to their increased potency. In general, they have a higher binding affinity for the GnRH receptors, are more resistant to enzymatic degradation, and/or have a longer half-life in the circulation. Following commencement of treatment with GnRH or its agonists, there is a transient period of increased gonadotropin secretion before the suppressive effects of pituitary desensitization are realized (Conn and Crowley 1991). This results in a delay of effect in both sexes. In females, this initial increase in gonadotropin secretion may induce estrus and ovulation, depending upon the reproductive status of the animal when treatment is begun. However, if a female were bred during this induced estrus, the continued administration of GnRH would likely terminate the pregnancy.

In males, there seem to be species differences in the degree of desensitization possible in response to GnRH agonists (see review by Vickery [1986]). Depending upon the species, pituitary desensitization is not necessarily accompanied by a decline in testosterone secretion and a suppression of spermatogenesis. However, when it is, testosterone supplementation may be necessary if maintenance of normal sexual behavior in males is desired (Vickery et al. 1984). Given these shortcomings, GnRH agonists may not be useful as male contraceptives. Nevertheless, they may have some application in control of androgen-stimulated aggressive behavior.

In Hawaiian monk seals (Atkinson et al. 1993) and free-ranging African elephants (Brown et al. 1993), single injections of GnRH agonists have been tested for their ability to suppress testicular function, i.e. testosterone production, thereby controlling aggressive behavior. Male Hawaiian monk seals may exhibit a breeding behavior called "mobbing" when their numbers exceed those of the females by more than 2:1. The "mobbed" female or immature seal is severely injured and often dies (Atkinson et al. 1993). Atkinson and coworkers found that after a transient increase, serum testosterone concentrations were reduced to castrate levels for approximately 2 months in male monk seals following a single injection of a GnRH agonist. Effects on sexual and aggressive behavior could not be measured because no female seals were available.

In the case of African elephants, males go into musth once or twice a year, during which time they are dangerously aggressive. Captive elephants in musth have injured and killed handlers (Brown et al. 1993). A single injection of a GnRH agonist caused an initial increase in serum LH and testosterone concentrations followed by a decline to baseline values. The one bull which was in musth at the time of treatment did not appear to be in musth after the decline in serum testosterone levels. Subsequent challenge with an intravenous injection of GnRH resulted in an attenuated LH response, suggesting partial desensitization of the pituitary. However, testosterone secretion was increased compared with controls, indicating a hypersensitivity to increases in GnRH-induced LH concentrations (Brown et al. 1993).

From these studies it appears that GnRH agonists show promise as agents that may decrease aggressive behavior by reducing serum concentrations of testosterone. This may be very useful in captive populations such as those in zoos. Yet it is important to note that some species, such as cattle, may respond to chronic treatment with GnRH agonists with an increase in testicular function, despite depressed pituitary function, as evidenced by elevated serum testosterone concentrations (Melson et al. 1986).

Another possible outcome of prolonged administration of GnRH agonists is the stimulation of both pituitary and testicular function, as described by Lincoln (1987). In that study, red deer stags received continuous infusion of a GnRH agonist for 72 days beginning after the rut in winter, a time when the testes are still secreting significant amounts of testosterone. It was expected that testicular activity would be suppressed, causing the stags to cast their antlers prematurely. In fact, treatment with the agonist resulted in increases in plasma LH and testosterone concentrations, testes growth, and aggressive behavior, and did not affect time of antler casting. The wide variation in response of the hypothalamic–pituitary–gonadal axis to exogenous GnRH may be due to several factors, including (1) choice of agonist, (2) dose, (3) treatment regimen, (4) reproductive status of the animal, and (5) species. Clearly more research is needed to determine the usefulness of this approach.

It has been well documented that continuous treatment with GnRH will suppress gonadotropin secretion in females (Nett et al. 1981, Adams et al. 1986, Khalid et al. 1989). Inhibition of ovulation caused by chronic administration of GnRH agonists has been successful in several species, including dogs (Vickery et al. 1989), cattle (Herschler and Vickery 1981), sheep (McNeilly and Fraser 1987), horses (Montovan et al. 1990), stumptailed monkeys (Fraser et al. 1980, Fraser 1983), and macaques (Fraser et al. 1987). We recently attempted to inhibit secretion of LH in white-tailed deer does *(Odocoileus virginianus)* by continually infusing a GnRH analog (Histrelin™), with the goal of preventing ovulation (Becker and Katz 1995).

Briefly, four does received Histrelin at 8.3 μg/hour subcutaneously via osmotic minipump, for 14 days during the breeding season. Controls were administered continuous saline infusions ($n = 3$). On Day 1 (Day 0 = day of minipump insertion), the Histrelin-infused group had a higher mean serum LH concentration than the control group (16.0 ± 5.3 *v.* 0.9 ± 0.4 ng/mL, respectively). By Day 2, mean LH concentrations did not differ between the groups and remained at baseline for the duration of infusion (fig. 2). On Day 10, both groups received a subcutaneous

injection of 100 μg Histrelin to test the ability of the pituitary to respond to additional stimulation. At 4 hours after injection, the mean serum LH concentration for controls was 17.8 ± 3.3 ng/mL and was still elevated at 10 hours. In contrast, serum LH concentrations in the Histrelin-infused group remained at baseline (0.5 ± 0 ng/mL) (fig. 3).

Apparently, continuous infusion of Histrelin caused pituitary desensitization. It was not possible to monitor the ovaries ultrasonically; however, serum progesterone concentrations did not indicate that any of the four does infused with Histrelin ovulated in response to the initial rise in serum LH concentrations. Further research is needed to determine if reproductive status influences whether or not ovulation is induced (an undesirable side effect) during the transitory increase in serum gonadotropin concentrations. The practicality of this approach is dependent upon development of a long-acting, slow-release preparation of agonist that can also be remotely delivered.

GnRH Antagonists

Pituitary suppression may be achieved by administration of antagonists of GnRH, which exert their effects by competing with endogenous GnRH, preventing sufficient GnRH occupation of receptors to stimulate gonadotropin secretion (Conn and Crowley 1991). The main advantage to using GnRH antagonists rather than agonists is that pituitary suppression is immediate. There is no initial increase in gonadotro-

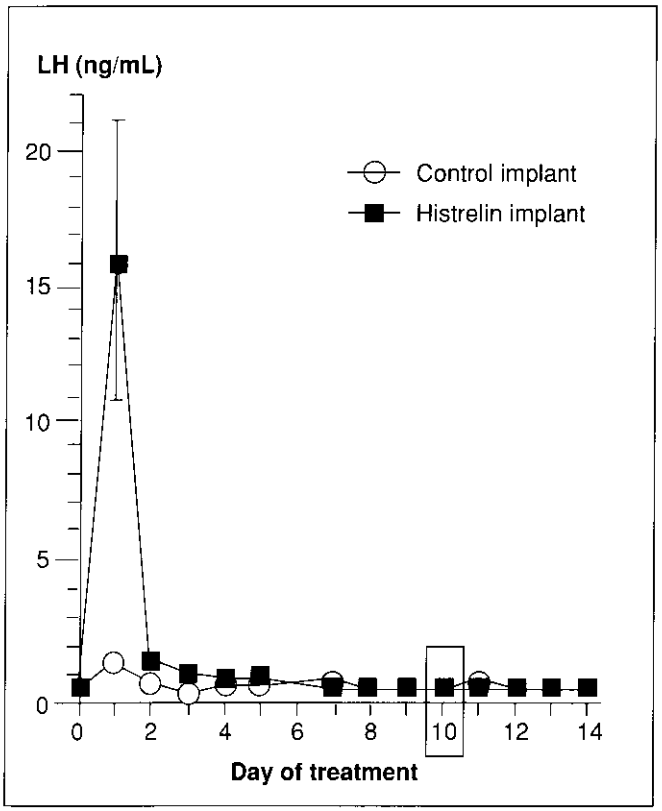

Figure 2. Effects of continuous administration of Histrelin (8.3 μg/hour, subcutaneously; $n = 4$) or saline (control; $n = 3$) on daily mean serum LH concentrations. Box indicates day of Histrelin challenge.

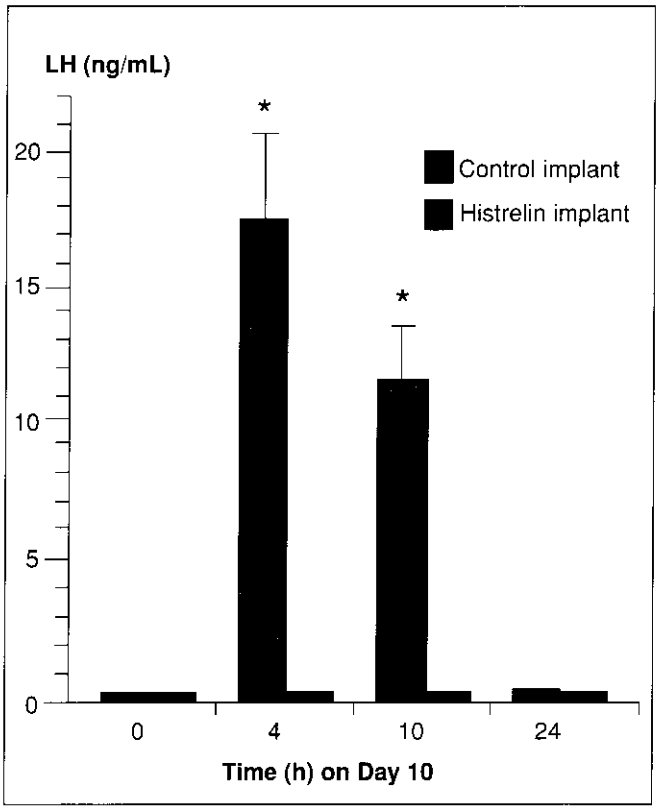

Figure 3. The LH response to a subcutaneous injection of 100 μg of Histrelin on Day 10 of the continuous Histrelin ($n = 4$) or continuous saline ($n = 3$) infusion period (Day 0 = day of implant insertion). * denotes treatment difference ($P < 0.05$).

pin secretion, which may stimulate the gonads. Unfortunately, these drugs are more expensive and require a higher dosage than the agonists, so they are best used for short-term treatment or instances where agonists are not effective (Vickery 1986). In males of several species, including rats, dogs, and monkeys, treatment with GnRH antagonists results in a decrease in serum LH and testosterone concentrations within hours, and that ultimately halts spermatogenesis (Vickery 1986). Choice of antagonist may be important, as evidenced by the work of Brown et al. (1993). They gave a single intramuscular injection of an antagonist to African elephant bulls that resulted in reduced basal and GnRH-stimulated serum LH and testosterone concentrations on Day 2 after injection. One of the bulls was in musth at the time of treatment but was no longer in musth by Day 2. In contrast, treatment of elephant bulls with a different antagonist of similar structure did not affect pituitary–testicular function, despite a higher dosage.

Antagonists of GnRH have successfully inhibited LH secretion and prevented ovulation in several species, including cattle (Rieger et al. 1989), rats, dogs, monkeys, and humans (see review by Vickery [1986]). For example, weekly subcutaneous injections for 20 weeks beginning during the midluteal phase of the estrous cycle resulted in suppression of circulating LH concentrations (compared with controls), and inhibition of ovulation throughout the treatment period in marmoset monkeys (Hodges et al. 1992). This effect proved to be reversible. Despite these successes, fertility control for wildlife often requires long-term treatment, for which GnRH agonists are better suited.

Immunoneutralization of GnRH

Another approach to inhibit gonadotropin secretion from the pituitary involves active immunization of an animal against endogenous GnRH. Because GnRH is a low-molecular weight, naturally occurring peptide, it is a weak immunogen. It must be adsorbed to a large, inert particle, such as charcoal, or covalently bound to a carrier protein, such as a serum albumin, to enhance immunogenicity. The latter seems to provide more consistent responses and higher antibody titers (see review by Jeffcoate and Keeling [1984]). Development of detectable antibody titers in the serum requires many weeks following primary immunization.

Although booster immunizations are not essential for the production of high antibody titers (Adams and Adams 1992), boosters almost always raise the existing antibody titers (see review by Schanbacher [1984]). Once titers are raised, circulating GnRH is recognized and bound by the anti-GnRH immunoglobulins before it reaches the pituitary, thereby suppressing LH secretion and usually FSH secretion (although not always to the same degree) and leading to an impairment of reproductive function. The degree of dysfunction appears to be correlated to the GnRH antibody titer; that is, the higher the titer, the greater the suppressive effects on reproduction (Lincoln et al. 1982, Safir et al. 1987, Bailie et al. 1989). Unfortunately, immediate inhibition of reproductive function is not possible unless immunization against GnRH is passive (administration of GnRH antiserum rather than a GnRH conjugate functioning as an antigen). For example, injection of ewes with ovine GnRH antiserum approximately 10 hours prior to the LH surge prevented the surge and blocked ovulation (Fraser and McNeilly 1982). Yet passive immunization against GnRH is not a practical method of fertility control because the effects are not long-lasting (Fraser et al. 1984). Frequent injections of GnRH antisera are not only impractical but also pose a health threat to the animal (Schanbacher 1984).

Active immunization against GnRH has successfully suppressed gonadotropin secretion and gonadal function in a variety of species, including rats and rabbits (Ladd et al. 1988), pigs (Esbenshade and Britt 1985, Awoniyi et al. 1987), sheep (Clarke et al. 1978, Adams and Adams 1986), horses (Garza et al. 1986, Safir et al. 1987), and cattle (Robertson et al. 1982, Adams and Adams 1990, Adams et al. 1993). However, little work has been done to test the effectiveness of this approach for wildlife. Studies in which red deer stags were actively immunized against GnRH met with varying degrees of success (Lincoln et al. 1982, Ataja et al. 1992, Freudenberger et al. 1993).

Effects on reproductive parameters ranged from a slight suppression of plasma LH concentrations compared with controls but no significant reduction of plasma testosterone concentrations (Ataja et al. 1992) to a significant decrease in testosterone levels compared with controls, testicular atrophy, and premature casting of antlers (Lincoln et al. 1982). Differences in the carrier protein used and the timing of the primary immunization with respect to reproductive season may account for this variability. When male and female wild Norway rats were actively immunized against GnRH, 100-percent sterility was attained for both sexes. In the males, testosterone was nondetectable, and testes were approximately 90-percent atrophied up to 11 months after vaccination (see Miller, this volume). Although these results are promising and immunoneutralizing GnRH is less costly than treatment with either GnRH agonists or antagonists, there can be large variation in response due to individual differences in the development of antibody titers.

Conclusion

None of the GnRH-related fertility control methods described herein meet all of the criteria of the ideal contraceptive agent outlined previously. One problem that may apply to any method of contraception in wildlife is the lack of consensus on the percentage of animals that must be rendered infertile to bring about the desired reduction in herd growth rate. Also, logistical and economic issues pertaining to delivery systems must be addressed. Perhaps the greatest problem with GnRH contraception is the resulting suppression of sexual behavior, which may affect social behavior and, consequently, social structure. This problem can be overcome by steroid supplementation using implants, but then food-chain contamination and the need to capture the animals to administer the treatment become issues that must be considered. However, inhibition of androgen-stimulated aggressive behavior may be desired in certain venues, such as zoos. In addition, care must be taken to ensure that the contraceptive activity of GnRH analog treatment lasts throughout the breeding season to avoid young being born when environmental conditions are unsuitable for offspring survival. Treatment must abolish, not merely delay, the breeding season. Targeting GnRH function for contraception of wildlife meets four of the six criteria mentioned earlier for the ideal contraceptive agent. Treatment is reversible, suitable for remote delivery, and unable to contaminate the food chain. Additionally, single administration is possible for active immunization against GnRH (and will be possible for GnRH agonists following the development of long-lasting, injectable microcapsules). Gonadotropin-releasing hormone contraception should be further investigated for potential applications in wildlife management.

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