The use of GnRH agonists implants in bitches and queens

Utilização de implantes de agonistas do GnRH em cadelas e gatas

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Abstract

GnRH (gonadotrophin releasing hormone) is a key hormone of reproductive function in mammals; agonist forms have been largely developed, and data concerning their use in small animal reproduction are now abundant. GnRH agonists act by a two-step mechanism. First, their agonist properties on the pituitary will cause marked LH (luteinizing hormone) and FSH (follicle-stimulating hormone) secretion into the bloodstream, accompanied by an increase in the concentrations of sex steroid hormones. Then, in case of constant administration, GnRH agonists will lead to pituitary desensitization, and FSH and LH levels will collapse. These two effects have been widely documented, and these compounds have many potential benefits in a clinical context, capitalizing both on their stimulating and sterilizing effects.

Keywords: bitch, deslorelin, GnRH agonists, queen.

Resumo

O hormônio liberador de gonadotrofinas (GnRH) é um hormônio chave na função reprodutiva dos mamíferos. Formas agonistas têm sido amplamente desenvolvidas e atualmente existem muitas informações sobre sua utilização na reprodução de pequenos animais. Os agonistas do GnRH atuam por meio de um mecanismo que envolve duas etapas. Inicialmente, suas propriedades agonistas irão causar secreção marcante de hormônio luteinizante (LH) e folículo-estimulante (FSH) pela hipófise, acompanhado pelo aumento das concentrações dos hormônios esteróides sexuais. Em seguida, no caso de administração contínua, os agonistas do GnRH irão causar dessensibilização e as concentrações de LH e FSH irão declinar. Estes dois efeitos já foram amplamente discutidos e estes compostos possuem vários benefícios no contexto clínico, devido aos às suas características estimulantes e contraceptivas.

***Palavras-chave**: cadela, deslorelina, agonistas do GnRH, gata.*

Introduction

GnRH (gonadotrophin releasing hormone) is a key hormone of reproductive function in mammals: agonist forms have been largely developed and data concerning their use in small animal reproduction are now abundant. GnRH agonists act by a 2-step mechanism. Firstly, their agonist properties on the pituitary will cause marked LH (luteinizing hormone) and FSH (follicle stimulating hormone) secretion into the bloodstream, accompanied by an increase in the concentrations of sex steroid hormones. Then, in case of constant administration, GnRH agonists will lead to pituitary desensitization, and FSH and LH levels will collapse. These two effects have been widely documented and these compounds have many potential benefits in a clinical context, capitalizing both on their stimulating and sterilizing effects.

Use of GnRH agonists in chemical sterilization

In the bitch

Effect of cycle stage and age

Cycle stage and age are seen to affect the response to the primary stimulating effect of GnRH agonists. Rubion et al. (2006) implanted bitches aged 4.88 ± 0.32 months with nafarelin implants, but no induced estrus was noted. Trigg et al. (2001) noted the same for bitches aged 4 months, but the use of deslorelin implants in animals aged 7 months or more systematically induced estrus in the 1 to 2 months following implantation. Likewise, Inaba et al. (1998) injected leuprolide depot in 6 prepubescent one-year-old bitches and 5 showed induced estrus.

Regardless of which agonists were used, adult bitches in anestrus were seen to respond by induced

estrus (McRae et al., 1985; Trigg et al., 2001; Fontaine and Fontbonne, 2010). Its characteristics will be described in detail below but it is interesting to note already here that this estrus occurred regardless of the stage of the anestrus (early, mid, late anestrus; Volkmann et al., 2006a; Fontaine and Fontbonne, 2010).

Trigg et al. (2001) did not observe the same phenomenon when working with bitches in metestrus when progesteronemia was greater than 5ng/ml. However, these data were mainly obtained in experimental beagle bitches, and it is now clearly stated that oestrus induction may occur in some diestrous bitches with high progesterone levels, sometimes above 60ng/ml (Fontaine and Fontbonne, 2010). Certain bitches were also treated with deslorelin late during gestation (more than 30 days after ovulation). No induced estrus was observed and the bitches whelped normally (Wright et al., 2001).

Duration of action

In the study by McRae et al. (1985), no periods of estrus were observed in bitches for the 18-month treatment period following the induction of estrus by an implant. The bitches came on heat between 3 and 18 weeks after the end of this treatment with nafarelin, clearly demonstrating here again its reversible nature. The stage of the cycle at which the treatment is started would appear to have an impact. In the study by Trigg et al. (2001), following insertion of a 3 mg desorelin implant, bitches came on heat again on average 13.9 ± 1.9 months later when implanted in the anestrus stage, 14.6 ± 3.5 months later when implanted in the metestrus stage, and 20.4 ± 3.4 months later when implanted during gestation. When a 12 mg implant was used, estrus was pushed back 15.5 ± 1.7 months when implantation took placed in metestrus and 19.6 ± 2.5 months when the bitches were implanted while gestating.

Return to fertility

The return to fertility in bitches after chronic treatment with GnRH agonists has been verified. Here, Trigg et al. (2001) re-used 9 treated bitches for breeding purposes at the first estrus following implant insertion. Six bitches became pregnant. However, as their re-introduction into breeding did not include the monitoring of estrus or any determination of optimal mating time, it is possible that all the bitches were capable of reproduction. Recently, Fontaine and Fontbonne (2010) also reported pregnancies in bitches at the first estrus following treatment with 4.7mg deslorelin implants.

In the queen

Clinical parameters

Few clinical information were collected, particularly as concerns the appearance of induced estrus. Toydemir et al. (2008) reported that two queens showed estrus behavior after treatment with 9.4 mg desorelin implants. However, no data were provided concerning the time elapsed between implant insertion and this estrus, nor its duration. No vaginal smears or routine additional examinations were performed to evidence the estrus in study queens. An examination such as an ultrasound scan of the ovaries used to track follicle growth could provide some interesting information. As in the bitch, the stage of cycle at the time of treatment may play a critical role in the occurrence of induced oestrus. Indeed, Goericke-Pesch et al. (2009) reported that oestrus induction was probable following implantation in seasonal anoestrus, whereas in interoestrous queens, this was rather rare.

Hormonal parameters

Only the hormonal response to implant insertion has been studied, primarily by the assay of fecal estrogens in treated animals (Munson et al., 2001; Toydemir et al., 2008). The results showed that estradiol secretion in the feces increased in the week following the start of treatment with GnRH agonists. This response could be considered as induced estrus, but would need to be correlated with clinical data. Estradiol blood levels were low 30 days after the start of the treatment.

Prohaczik et al. (2008) suggested a different approach. The queens were placed with vasectomized males. In this way if they came on heat they could be mated and ovulation detected by an assay of progesterone, thus confirming induced estrus and the capacity to ovulate. Here again, the correlation with purely clinical criteria is missing given that a host of behavioral characters (males that refuse to mate, females that fail to cooperate with mating) can compromise the data. In this study, progesterone blood levels increased in two queens out of six after implantation, but it is not impossible that induced estrus affected all those concerned.

Duration of action

Munson et al. (2001) noted that queens receiving a 6 mg deslorelin implant did not show any increase in fecal estradiol for 8 to 14 months after implantation, suggestive of a fitting duration of action. However, one animal showed an estradiol peak 4 months post-implantation, showing that interindividual variations, like in the bitch, are very substantial. Toydemir et al. (2008) reported an increase in fecal estradiol concentrations 16.5 months after insertion of a 9.4 mg deslorelin implant. A queen in this study also showed an increase in blood estradiol levels 3.5 months post-implantation. This study lasted 18 months at the end of which all the animals were sterilized, and none of the animals presented any signs of being on heat over this period.

When 20mg nafarelin implants were used, the queens studied failed to show any progesterone elevations for a period of 2.5 years. This study lasted three years and none of the queens showed any signs of being in heat during this period (Prohaczik et al., 2008). However, the same remark as made above concerning individual animal behavior can again be made here.

Goericke-Pesch et al. (2009), using 4.7 mg deslorelin implants, observed that in queens duration of efficacy varied between 6 to 24 months.

Fertility parameters

In the study by Toydemir et al. (2008), the two queens that showed signs of estrus following implantation were mated several times but were diagnosed as empty by ultrasonography one month later. No assay was performed of blood progesterone and it is therefore impossible to state whether they had ovulated or not. However, it is possible - like in the bitch - that the luteal period, if any, is shortened by implantation. To the best of our knowledge this has never been studied. As none of the animals in the three studies was subsequently used for reproduction, no information is available describing the return to fertility in these individuals.

Histological parameters

At the end of their study, Toydemir et al. (2008) sterilized all the animals. The ovaries of treated queens did not show any follicular structures or corpora lutea, unlike the individuals in the control group. These ovaries were also atrophied in comparison with the control group. It would have been of interest to obtain more detailed information concerning the type of follicles (primordial, primary, secondary) encountered in these ovaries. The logical follow-up to such a study would be to examine the ovaries and determine the time required for treatment reversibility.

Capitalizing on the stimulating effects of GnRH agonists: oestrus induction.

This indication has been explored only in bitches; no such studies have been conducted in queens. However, estrus has been induced in wild felids using deslorelin implants, namely in female cheetahs and lions (Fontbonne et al., 2007).

Time between treatment initiation and the induction of estrus

The time between treatment start and the onset of estrus is fairly constant for all protocols. Cain et al. (1989), using infusion pumps delivering GnRH 140ng/kg every 90 minutes for 11 to 12 days, obtained estrus after 5.6 ± 0.53 days in all test bitches. Inaba et al. (1998) , using a leuprolide depot injection of 1 mg/kg, obtained estrus in all 12 bitches in their study on average 10.3 ± 0.9 days post-treatment. Concannon et al. (2006), using lutrelin, induced estrus in 89% of treated bitches 4.8 ± 0.2 days after the start of the treatment. All the bitches in the study by Kutzler et al. (2002), who used a 2.1mg deslorelin implant, came into heat at the latest 6 days after the start of the treatment, with estrus generally appearing in the 3 to 5 days following implantation (Kutzler, 2005; Volkmann et al., 2006a). Recently, Fontaine and Fontbonne (2010) obtained similar results with 4.7 mg deslorelin implants, with heat induction occurring 4.2 ± 1.4 days post-implantation.

Ovulation

Most of the treated bitches ovulated after induced estrus, but here again the results differ from one study to another. Whereas Concannon (1989) obtained only 18 ovulations out of 24 bitches treated in his protocol with lutrelin in his first study and only 59% ovulations in the second (Concannon et al., 2006), the results obtained in other studies are more encouraging. All the beagle bitches treated with deslorelin by Kutzler et al. (2002) ovulated. Seven bitches ovulated between 11 and 15 days after implantation, and 4 after about 11 days. Volkmann et al. (2006a), also using deslorelin implants, obtained similar results: the time between implantation and peak LH levels ranged from 9 to 17 days, indicating that ovulation occurred between 11 and 19 days after implantation. 76% of the 29 bitches implanted by Fontaine and Fontbonne (2010) with deslorelin implants

ovulated, this ovulation occurring 12 ± 2 days post-implantation.
Inaba et al. (1998) and Cain et al. (1989). did not determine ovulation time, but simply used the bitches for reproduction. However, the pregnancy rates obtained (14/18 for Inaba et al.,1998; 7/8 for Cain et al., 1989) in these two studies confirm that the bitches had the capacity to ovulate.

Luteal function

The constant administration of GnRH agonists results in hypoluteidism caused by gonadotrophic insufficiency. In this case gestation may not be continued to term and the luteal phase ends between 30 and 40 days after the treatment is started(Volkmann et al., 2006b; Fontaine and Fontbonne, 2010).

To avoid this phenomenon, Kutzler et al. (2002) suggested removing the 2.1 mg deslorelin implant used in their study after the rise in blood progesterone levels, around the LH peak. On the other hand, Fontaine and Fontbonne (2010) suggested to remove the implant after ovulation took place, as they experienced anovulatory cycles when using the former suggestion. The implant was inserted beneath the vulvar mucosa, in reference to practice in mares (Kutzler et al., 2002), or in the ombilical area (Fontaine and Fontbonne, 2010) where it remained, without migration, and was therefore easy to locate and remove. Five bitches treated in this manner became pregnant out of the 8 in the study of Kutzler et al. (2002), whereas Fontaine and Fontbonne (2010) obtained pregnancy in 65% of the treated bitches (n = 29). However, both studies reported lost of litters during pregnancy, one between 30 and 37 days postovulation (Kutzler et al., 2002) and one 58 days post ovulation (Fontaine and Fontbonne, 2010).

Subsequently, (Volkmann et al., 2006b) noted that treated individuals absorbed most of the agonist contained in 2.1 mg deslorelin implants in the 10 days following implantation. Implant removal therefore was of little practical use. The bitches induced in this manner saw their blood progesterone levels decrease significantly 35 days later, which may explain why the luteal phase in these induced bitches was not conducive to the maintenance of gestation. Following of the luteal phase is therefore recommended after this kind of treatment, to ensure adequate progesterone replacement therapy if needed (Fontaine and Fontbonne, 2010).

The use of preparations for injection could prove to be more practical. However, the dose-duration effect should in this case be carefully controlled for although Inaba et al. (1998)obtained 14 pregnant bitches out of 18 with leuprolide 1mg/kg, (Kutzler, 2005) obtained controversial results with a deslorelin preparation for injection. Whereas 3 pregnancies out of 3 were obtained in Lakeland terriers following deslorelin 1.5 mg i.m., the 9 beagle bitches treated in the same fashion in an additional study did not even express estrus (Kutzler et al., 2006).

Fertility results

The compound used also appears to have an impact on fertility results. For instance, whereas the studies conducted by Concannon with lutrelin gave poor results (9 pregnant bitches out of 24 induced; Concannon, 1989; 44% gestation; Concannon et al., 2006), the use of leuprolide 1mg/kg (14 gestations out 18 bitches treated; Inaba et al., 1998) and 2.1 mg deslorelin implants (5 pregnant bitches out of 8 treated; Kutzler et al., 2002); 9 gestations out of 13 bitches included in the study; Volkmann et al. 2006a) opens up interesting perspectives.

The stage of the cycle at which induction takes place also may play a role. With leuprolid, Inaba et al. (1998) obtained 6/6 pregnancies in bitches induced 150 days post-partum, whereas induction 120 days post-partum resulted in pregnancy in only 3 cases out of 6. Fontaine and Fontbonne (2010) also reported that bitches that presented anovulatory cycles after heat induction using deslorelin had been mainly implanted in early anoestrus, whereas those implanted in late anoestrus ovulated.

Volkmann et al. (2006b)attempted to trigger fertile estrus in bitches in metestrus by inducing luteolysis with prostaglandins then using 2.1 mg deslorelin implants: although they managed to induce estrus in all the bitches, only 4 out of 10 ovulated and only one started pregnancy: the embryo was resorbed prior to term.

These findings confirm that after metoestrus in the bitch, a period of uterine endometrial regeneration is necessary both for implantation and for gestation to term. Although certain studies indicate that the endometrium is fully involuted 135 days after the end of the luteal phase (Anderson and Simpson, 1973), these findings are relatively old and need to be confirmed. It nevertheless clearly appears that inducing estrus 150 days after the end of the luteal phase gives better results in terms of fertility. Use of GnRH agonists in a therapeutic framework**.**

Preventing the initial stimulating effect

The stimulating effect of GnRH agonists, as already seen above, is more pronounced in the female and leads to the onset of estrus. Since Trigg et al. (2001) noted that if bitches with blood progesterone levels in excess of 5 ng/mL are treated with deslorelin implants, they do not express induced estrus , this would appear to indicate the use of progestogens. However, the results obtained are fairly controversial. Whereas Wright et al.

(2001) noted that megestrol acetate was able to prevent the induced estrus, Corrada et al. (2006) observed estrus in 3 bitches between 26 and 51 days after implantation: the treatment with progestagens simply time-shifted the induced estrus. However, when starting the treatment 4 days before implantation, the same team obtained estrus in only 10% of the bitches (Corrada et al., 2006). Sung et al. (2006) attempted the same experiment by starting the treatment 7 days before implantation, but 4 of the 5 treated bitches expressed estrus. The time elapsed between implantation and the expression of estrus ranged from 3 to 19 days with a mean of 8.4 ± 1.7 days. The authors also observed that although the treatment with progestogens effectively decreased the pituitary response in implanted bitches (the bitches treated in this manner showed significantly lower LH concentrations than untreated animals) an increase occurred in the ovary response to deslorelin stimulation (the treated bitches showed significantly higher blood estradiol levels). Recently, Valiente et al. (2009) combined the GnRH antagonist acycline in deslorelin-implanted anoestrous bitches to prevent oestrus induction: initial ovarian stimulation was prevented in one quarter of the treated bitches, whereas the stimulation period was postponed and ovulation was inhibited in approximately half of the remainder.

Today therefore, no satisfactory protocol has been devised to inhibit the appearance of induced estrus. Some authors had recommended to administer GnRH agonists therapy during diestrus (Romagnoli et al., 2009), but this should not be effective in all cases, as heat induction was reported in some individuals administered with deslorelin implants when progesterone levels were above 60 ng/mL (Fontaine and Fontbonne, 2010). This is an obstacle to the use of these compounds both for the sterilization of females and for therapeutic purposes.

Use in the treatment of sex hormone-related conditions

Mammary tumours

Lombardini et al. (1999) showed that goserelin 60 mg/kg given every 3 weeks reduced the size of mammary tumors after 3 months in 9 study bitches. The same team also investigated the effects of goserelin on bitch mammary cancer cells cultured *in vitro* (Pagnini et al., 2002) and showed that the agonist was also able to inhibit the mitogenic effects of a growth factor, EGF (Epithelial Growth Factor) which promotes the growth of mammary tumors. This effect on the tumors may be related to a direct effect by the agonist on the tumor cells, not a spaying effect. However yet, not enough data concerning the use of GnRH agonists as an adjuvant in the treatment of canine mammary tumors are yet available.

Urinary incontinence

Urinary incontinence in the bitch is a complication which can arise after spaying and may affect up to 20% of cases following a decrease in urethral sphincter competence.

The hypothesis put forward today is that this sphincter incompetence is related to an increase - following gonad removal - in serum levels of LH and FSH which results from loss of the feedback control exerted by the gonads on the pituitary. Receptors for these gonadotrophins have been found in the bladder and urethra of male and female dogs, suggesting that they play a role in bladder metabolism (Ponglowhapan et al., 2007). However, their mechanism of action is still poorly understood and although a direct effect seems unlikely - Reichler et al. (2005) having shown that LH/FSH levels are significantly lower in incontinent spayed than in continent spayed bitches - an indirect effect through a decrease in the number of their receptors in the bladder and urethra is now envisaged. In support of this, it is known that LH and FSH participate in the metabolic processes that break down collagen in the bladder, and this decrease in the number of their receptors may be accompanied by an increase in collagen in the bladder, reducing its capacity to contract (Ponglowhapan et al., 2008).

The suppression of pituitary secretion by GnRH agonists therefore presents as a logical solution. Thus, leuprolide, triptorelin, buserelin and deslorelin (Reichler et al. 2003) have already been used to treat urinary incontinence in the bitch. Using leuprolide, 71% of treated bitches showed a clinical improvement, with 50% of these animals remaining continent for several months and one for even a year (Reichler et al., 2006b). In a second study, 12/13 bitches showed improved or restored continence for 1.5 months to 5 years (Reichler et al., 2003).

Recent studies have suggested that GnRH agonists may have a direct action on bladder function. Reichler et al. (2006a) noted that treatment based on leuprolide had no effect on urethral closure pressure whereas it did have an action on bladder function (mean bladder volume in these bitches increased from 109 to 172mL). As no relationship was found between serum gonadotrophin levels and urodynamic parameters, it is likely that the GnRH agonist exerted a direct effect on bladder function.

However, it is important to note that the results obtained with treatments based on alpha-agonists such as phenylpropanolamine, used as first-line treatment today in the bitch, are far superior (91.8% of bitches generally respond to the treatment; Reichler et al., 2006b) and GnRH agonists should be presented rather as alternatives in bitches where this treatment is contraindicated (aggressiveness, cardiac disorders). In certain cases

that response poorly to alpha-agonists, a combination with GnRH agonists may be able to restore continence. For instance, Reichler et al. (2003) noted that whereas 7/12 bitches showed an improvement in continence with GnRH agonists, their combined use with an alpha-agonist restored continence in all the animals.

Puppy coat syndrome

The increased plasma concentrations of gonadotrophins that follow surgical spaying cause changes in the anagen phase - telogen phase ratio of the hair follicles. Fully 20% of dogs then show a change in their coat which is known as "puppy coat syndrome".

GnRH agonists improve coat quality in 79% of all cases treated. Although it is known that this improvement is associated with an increase in the hair catagen phase, without any change in the anagen-telogen ratio, the pathophysiological mechanism underlying this change in animal coat has not been fully elucidated (Reichler et al., 2008).

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