Results of GnRH Agonist Implants in Oestrous Induction and Oestrous Suppression in Bitches and Queens

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Contents

Over the last 10–15 years, long-acting GnRH agonists have become widely available. In the field of small animal reproduction, most recent studies have focused on the use of two compounds developed under the form of subcutaneous implants: azagly-nafarelin and deslorelin. Only the latter has been commercially available for use in male dogs, first in Australia and New Zealand, then in several countries of the European Union since 2008. Although officially marketed for male dogs, this compound has also been studied in bitches and more recently in queens. Some published papers or recent presentations at congresses still unpublished – have focused on the use of GnRH agonists implants in females.

Oestrous Induction in Bitches

The rate of oestrous induction after the administration of a subcutaneous GnRH agonist implant in anoestrous bitches is very high and oestrus occurs fast after implantation. The differences among authors are related to time to remove the implant, ovulation rate and fertility of the induced oestrus. The use of 2.1-mg implant of deslorelin (Ovuplant®; FortDodge Animal Health, Overland Park, KS, USA), used to induce ovulation in mares, was able to induce oestrus in all female beagles, with proestrous signs generally appearing approximately 6 days following implantation (Kutzler et al. 2002). Oestrus was induced in all bitches by Ovuplant® regardless of the site of implantation (vestibular submucosa vs. subcutaneous) or dose 2.1 mg vs. 1.05 mg (Kutzler et al. 2002; Volkmann et al. 2006b; Kutzler et al. 2009). The time of appearance of serosanguinous vulvar discharge was similar using 4.7-mg deslorelin implants (Suprelorin®, Virbac, Carros, France) in two recent studies. Fontaine et al. (2011a) using 32 bitches of different breeds observed vaginal discharge in average 4.3 ± 1.4 days and in the study by Walter et al. (2011) using 11 beagles, the average was 5.6 ± 0.6 days after implantation. Ovulation appeared sooner and with less variation of occurrence during heats, in average 11.8 ± 2.1 days after implantation.

Although oestrous induction seemed to happen at a high rate and very constantly among authors, ovulation rate was less consistent. Using the Ovuplant® implant, ovulation rate ranged from 62% to 100% depending upon the time of the oestrous cycle of the bitches when they were induced, dioestrus or anoestrus, with higher rates during anoestrus (Kutzler et al. 2002; Volkmann et al. 2006b; Kutzler et al. 2009). In the study by Fontaine et al. (2011a), ovulation was reported in 87.5% of bitches when implanted in late anoestrus (200–590 days following their previous oestrus), while it occurred only in 62.5% of bitches implanted in early anoestrus (80–160 days following their previous oestrus). In the study by Walter et al. (2011), all implanted bitches ovulated, although the stage of anoestrus of these bitches was not specified.

The necessity of removal of the implant to avoid subsequent hypoluteoidism caused by gonadotrophin insufficiency is still questionable. Some unpublished personal observations showed that some bitches were able to deliver successfully, although the implant had not been removed. However, when the implant was not removed, the percentage of late abortions was above 65 (Kutzler et al. 2002; Kutzler et al. 2009). To avoid this phenomenon, implants were placed in an area where they can be easily removed, the preferred sites of implantation being the post-umbilical area or the medial side of the leg. Time of implant removal varied also depending on the authors, at the beginning of clinical heats, around the time of the LH peak or around the time of ovulation. Even with the removal of the implant, luteal failure was suspected in five bitches in the study of Fontaine et al. (2011a), three of them were pregnant, two were successfully supplemented with micronized progesterone and gave birth normally, while the third one was not supplemented and gave birth prematurely 58 days after ovulation. The reason of the late abortion is most likely associated with lower levels of progesterone in the induced oestrus (Volkmann et al. 2006a; Fontaine et al. 2011a). The fertility results differed among studies and treated groups and varied between 25% and 69.6%. Some factors were suspected to play a role in these differences such as the stage of the cycle of implanted bitches, the time of implant removal or time of ovulation in relation to the time of implant insertion. Yet, in many studies, the fertility of the induced oestrus was above 65% (Volkmann et al. 2006b; Fontaine et al. 2011a; Walter et al. 2011), values that are similar to the ones observed in spontaneously cycling anoestrous bitches (Volkmann et al. 2006b) and similar to the results reported by Fontaine et al. (2011a). It is not easy to explain why Walter et al. (2011) showed better ovulation and pregnancy rates than in the study by Fontaine et al. (2011a). However, Walter et al. (2011) only worked with beagles and the implants were removed after the onset of oestrus, which was earlier than in the study by Fontaine et al. (2011a). In addition, Walter et al. (2011) ovariohysterectomized the beagles at 19 days post-ovulation after
confirming pregnancy instead of letting the pregnancies continue to term.

**Oestrous Prevention in Adult Bitches**

**Prevention of induced oestrus after implantation**

One of the major undesirable effects when using the 4.7-mg deslorelin implant to suppress sexual activity is the immediate induced oestrus in anoestrous adult female dogs. When the implant is placed in anoestrous bitches, almost 100% express signs of oestrus. Prevention of this induced oestrus has been attempted through different ways with controversial results. Trigg et al. (2001) and Romagnoli et al. (2009) recommended using the implant in dioestrous bitches because no induction of oestrous signs was observed when progesterone concentration was above 5 ng/ml. However, this observation was not repeated in the study from Fontaine and Fontbonne (2010) where oestrous signs were reported after the implantation of some dioestrous bitches, even when progesterone concentration was above 60 ng/ml. Wright et al. (2001) noted that megestrol acetate (MGA) at a dose of 2 mg/kg/day for 14 or 21 days, starting 1 week prior to the administration of a 6-mg deslorelin implant, was able to prevent the induced oestrus in anoestrous bitches. Corrada et al. (2006) used the same dose of MGA but for a shorter duration of treatment (8 days), starting 4 days prior to placing a 10-mg implant of deslorelin in 42 bitches. The initial ‘flare-up’ effect was inhibited in 90% of bitches but a postponed induced oestrus at 26–51 days after implantation was observed. This suggests that the treatment with progestins only time-shifted the induced oestrus, which limits the use of this protocol.

In the study of Sung et al. (2006) using the same methodology of Wright et al. (2001), with Greyhound bitches in late anoestrus, only 20% of the female dogs did not express oestrous signs. The prevention of induced oestrus might be more difficult to obtain in late vs. early anoestrus. Valiente et al. (2009) used acycline, a GnRH antagonist in anoestrous bitches in order to prevent the deslorelin-induced oestrus. A 10-mg implant of deslorelin was placed with a single injection of acycline (330 µg/kg) subcutaneously in the 48 h following implantation. The induced oestrus was only suppressed in 25% of the bitches with half of them ovulating. In the study from Fontaine et al. (2011b), implanted adult bitches were concomitantly treated for 15 days per os with the aromatase inhibitor anastrozole 0.1 mg/kg (Group 1, n = 3, Arimidex®; AstraZeneca, Paris, France) or the anti-oestrogen clomiphene acetate 5 mg/kg (Group 2, n = 8, Clomid®; Sanofi-Aventis, Paris, France). In Group 1, two bitches presented bloody discharge and keratinization of the vaginal epithelium after 5–6 days post-implantation. Ovulation was confirmed in these two bitches. In Group 2, no bloody discharge was observed in 6/8 bitches, but keratinized cells were observed in vaginal smears of all bitches. Ovulation occurred in 5/8 bitches between 16 and 18 days post-implantation.

Another approach (Anjolras 2011) attempted recently in our laboratory to prevent the ‘flare-up’ effect of the deslorelin implant was the use of osaterone acetate (Ypozane®; Virbac, Carros, France) around the time of implantation. Osaterone acetate was administered orally at a dose of 0.5 mg/kg for 2 days consecutively, and on the last day of treatment, the implant of 4.7 mg of deslorelin was placed in 16 anoestrous bitches of different breeds, aged from 6 months to 9 years. This treatment prevented oestrous signs in 81.3% of the bitches, but 12.5% of them presented silent heats and another 12.5% had anovulatory cycles. Yet, the prevention of both oestrous signs and ovulation was only achieved in 68.7% of the bitches. No postponed oestrus was observed because bitches were followed for over a year to evaluate the duration of chemical spaying.

Return to oestrus was observed in three of 16 bitches at a mean of 253.3 ± 96.9 days.

The mechanism involved in the suppression of the deslorelin-induced oestrus through progestins is unclear, but efficacy appears dependent on dose and duration of treatment. Reduced responsiveness of the pituitary gland to deslorelin combined with an inhibition of follicular response to gonadotrophin has been suggested in progestin-treated animals (Wright et al. 2001; Sung et al. 2006). Owing to the wide variation between individual responses, these compounds cannot be considered at the moment as valuable alternatives to repeatedly prevent the induced oestrus occurring in anoestrous bitches. Therefore, the prevention of induced oestrus after the implantation of GnRH agonists in adult bitches remains to be further studied.

**Eventual side effects in implanted bitches**

Possible side effects described in 27.6% (8/29) of the bitches included one or more of the following: induced lactation, persistent oestrus, ovarian cysts, depression and cystitis (Anjolras 2011). In the study from Fontaine and Fontbonne (2010), using 4.7 mg SC implants, 7/47 bitches presented prolonged heats (including two cases of ovarian cysts), 5/47 had an induced lactation, 3/47 presented some behavioural changes and 8/47 miscellaneous problems (cystitis, vomiting, allergic reactions). The removal of the implant usually improved the bitch’s condition within 15 days. However, ovariectomy was necessary in six cases or was performed on the owner’s request in one case. Metropathies were observed in 11/80 bitches in a study by Palm and Reichler (2010), with or without preliminary signs of oestrus after implantation. According to these authors, old age or a history of receiving progestagenic treatment were predisposing factors for developing a metropathy. Recently, a case of induced ovarian cysts followed by a subsequent pyometra following implantation was published (Arlt et al. 2011).

**Duration of efficacy**

Oestrus was suppressed for up to 27 months with duration dependent on dose and the effect was reversible (Trigg et al. 2001; Sung et al. 2006). The stage of the cycle at which the treatment is started appeared to have no impact on the duration of oestrous suppression after implantation (Trigg et al. 2001). According to our
observations (work still under investigation), adult bitches implanted only once with a 4.7-mg deslorelin implant came in heat 10.2 ± 5.1 months after implantation (2.1–23.3 months).

Future fertility
Return to fertility after implantation is still unclear, although 6/9 bitches became pregnant at the following oestrus post-implantation (Trigg et al. 2001) and four of them gave birth to healthy litters. Unpublished observation still under investigation in our laboratory demonstrated good fertility rate and tended to show that bitches are further able to give birth naturally, with no sign of hypoluteoidism during pregnancy.

Oestrous Prevention in Prepubertal Bitches
Very few studies have been published using GnRH agonist implants in order to postpone puberty in female dogs. Five-month-old bitches implanted with azagly-nafarelin subcutaneous (SC) implants (Gonazon®; Intervet-Schering Plough Pharma Animal Health, Beau-couzé, France), which were removed 1 year after implantation, reached puberty at 25.5 ± 5 months of age (Rubion et al. 2006). Using a 4.7-mg deslorelin SC implant in 4-month-old bitches, no signs of oestrus were observed for the following 36 weeks by Trigg et al. (2006), but the exact date, clinical and hormonal features of the first oestrus after implantation were not mentioned. Interestingly, in the same study, the use of deslorelin implants in bitches aged 7 months or more systematically induced oestrus within 1–2 weeks. This confirmed that the age at implantation seemed to play an important role in the response to the administration of GnRH agonist implants.

We conducted a study to investigate the use of deslorelin SC 4.7-mg and 9.4-mg implants in the postponement of puberty in bitches of different breeds below 6 months of age (4.2 ± 0.6 months). Altogether, 15 bitches were implanted with a 4.7-mg implant between January 2010 and October 2010 and 11 bitches were implanted with a 9.4-mg implant between November 2010 and February 2012. The implants were administered subcutaneously in the post-umbilical region and left in place during the whole period of the study. Follow-up with the owners of the implanted bitches consisted in reporting any sign of oestrus or any side effect noticed. A clinical examination of the implanted bitches was performed at the end of the first oestrus. Possible side effects (aspect of the fur, growth...) were recorded, and ovulation was confirmed using a quantitative progesterone assay (Elecsys 2010; Roche Diagnostics, Mannheim, Germany). No bitch showed any signs of induced oestrus soon after implantation. No clinical side effect was recorded by the owners. During the time of the study, eight of 15 bitches implanted with the 4.7-mg implant showed their first oestrus 13, 14, 15, 17, 20, 21, 23 and 24 months post-implantation at the end of the study. They all showed a high plasma progesterone level (>15 ng/ml) at the end of the first oestrus, in accordance with a recent ovulation. One Golden Retriever bitch implanted with a 4.7-mg implant presented some doubtful signs of urinary incontinence 8 months after implantation. The bitch was unsuccessfully treated with phenylpropanolamine (Propalin®; Intervet, Boxmeer, Netherlands) and was spayed at the request of the owner 12 months after implantation, before any sign of oestrus was observed. The urinary signs surprisingly ceased soon after spaying. We cannot explain this outcome as it appears counterintuitive because urinary incontinence is usually observed in spayed bitches (oestrogen-responsive urinary incontinence). The six remaining bitches did not show any sign of oestrus at the end of the observation period. They had been implanted between 16 and 25 months before. No effect of the size of the breed could be suspected in our study. None of the bitches that underwent puberty showed any abnormality in the fur (such as puppy coat), in growth or in the development of the external genital organs. Among the 11 bitches that were implanted with a 9.4-mg implant, no bitch showed any sign of oestrus by the end of the study. They all had been implanted for 8–15 months.

Oestrous Induction and Prevention in Queens
The use of deslorelin in the queen has been predominately to prevent oestrus. The mechanism of action seems to be similar to the bitch. Nevertheless, the flare-up effect is poorly described and inconsistent. Although signs of oestrous behaviour were reported after implantation, most of the time no vaginal smears or additional ultrasonographic examinations were performed to evidence the oestrus in the studied queens. In two studies, oestriadiol secretion in the faeces increased within a week following the treatment with GnRH agonists (Munson et al. 2001; Toydemir et al. 2012). However, oestrous behaviour was observed only in two queens in a group of 14 treated with 9.5-mg deslorelin implants (Toydemir et al. 2012). In the presence of vasectomized males to evaluate oestrous/ovulation rate soon after the insertion of the implant, progesterone rise was observed only in two of six queens, but no oestrus or mating behaviour was recorded (Prohaczik et al. 2008). When the implant was placed during interoestrus, an initial increase in oestriadiol was first observed, followed by a decline while progesterone concentrations were rising, indicating ovulation induction as well (Goericke-Pesch 2011). The induction of the oestrus by deslorelin in the queen may have been affected by the time of the cycle when the implant was placed as has been observed in bitches. All queens that received the implant during seasonal anoestrus showed increased levels of oestrogen when only a few during interoestrus (Goericke-Pesch et al. 2010).

To our knowledge, no study aiming to determine the ovulation rate and fertility after oestrous induction with deslorelin implants in queens has been published so far. No pregnancy was reported in the two queens with high progesterone levels post-implant by Prohaczik et al. (2008) or in the two queens mated by multiple tomcats by Toydemir et al. (2012).

The efficacy and safety of oestrous suppression with deslorelin in queens has been demonstrated in many
studies (Munson et al. 2001; Prohaczik et al. 2008; Goerricke-Pesch et al. 2010; Ackermann et al. 2012; Toydemir et al. 2012). However, the duration of the suppression is very variable. By detecting oestrus in the faeces, the effect of GnRH agonists was 8–14 months and more than 18.5 months in queens receiving a 6.0-mg and 9.5-mg deslorelin implant, respectively (Munson et al. 2001; Toydemir et al. 2012). One queen in each study showed increased levels of oestradiolemia approximately 4 months, indicating the individual effect in the response of the females. When observation of oestrous behaviour was used to measure the efficacy of oestrous suppression, the duration was superior to 3 years (Prohaczik et al. 2008) and varied between 6 and 24 months with 4.7-mg deslorelin implants (Goerricke-Pesch et al. 2010; Goerricke-Pesch 2011). When the time of oestrous suppression was controlled, and the implant was removed after 2 months only, the return to cyclicity was less variable and the average time to return to cyclicity was 42 days (Ackermann et al. 2011), but the study was conducted in four cats only.

Although the data about further fertility after oestrous suppression are scarce, there is some unpublished information of queens able to reproduce after being implanted; however, no study has been carried out to evaluate this subject. Ackermann et al. (2012) using deslorelin implant in a limited time of 90 days demonstrated the viability of oocytes post-implantation. 10 queens were submitted to an oestrus and ovaulation induction protocol with eCG and hCG after oestrous suppression, 100% respond to the ovulation protocol and all recovered oocytes were viable. Yet, fertility was not assessed in this study.

Deslorelin implants may also be used to postpone puberty in the queen. The implant should be placed when the female has reached 50% of the body weight. Deslorelin did not affect the growth rate and increased the age of puberty in average from 177.8 ± 10.6 to 281.2 ± 21.5 (Risso et al. 2012).

Conflicts of interest

The experimental work with deslorelin (Suprelorin®) done by the CERCA group at the Alfort Veterinary College was financially supported by Virbac. K. Reynaud has no conflicts of interest to declare.

References


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