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Update on medical control of reproduction in bitch with a focus on deslorelin implant

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Abstract

Medical control of reproduction in the bitch is a huge challenge. Currently, only progestins are officially marketed for this purpose but they have undesirable effects. Subcutaneous implants containg GnRH agonist deslorelin have been released in the European market. They may be used 'off-label' to prevent cyclicity in adult bitches. A 4.7 mg deslorelin implant (after an initial 'flare-up' in increases in pitutary release of gonadotropins [FSH and LH] and subsequent downregulation) prevents estrus for ~ 10 months on an average, with large individual variations. The major disadvantage of using deslorelin implants in adult bitches is the initial induction of estrus soon after implantation. Currently, there are no 100% satisfactory protocols aviable to prevent this effect. Additionally, deslorelin treatment may sometimes cause side effects (e.g. prolonged estrus, ovarian cysts, prolonged lactation or even and rarely uterine disease). Ultrasonographic examination of ovaries and uterus is mandatory to ensure that there are no pathologies (e.g. ovarian cysts or cystic endometrial hyperplasia) present prior to treatment. If the bitch does not develop any side effects in the first weeks after first implantation, she can be reimplanted regularly several times and even life-long without any subsequent problem. A single treatment with a deslorelin implant does not compromise fertility of the bitch in subsequent estruses. Treatment of prepubertal bitches before 6 months of age is useful to prevent induced estrus. However, it delays puberty and therefore prevents external genital development during treatment, but it does not seem to have any subsequent effect on growth or health. Future approaches of contraception in bitches are in use of aromatase inhibitors, immunocontraception, especially antiGnRH immunization, drugs counteracting the effects of kisspeptins, and genetically mediated contraception.

Keywords: Bitch, contraception, progestins, deslorelin, kisspeptins, immuno-contraception

Introduction

The nonsurgical control of reproduction in the bitch is a huge challenge and has a potentially large market in a near future. In Europe, many owners fear surgical approach and consider ovariectomy as mutilation. Progestins are the only officially approved drugs in Europe for this purpose, but they have many undesirable effects. Development of medical contraception is however extremely complex because it should, in theory, be used in companion animals as well as in stray dogs. However, the expectations are very different in each case. This article presents the main prospects for medical contraception of bitches.

Progestins

There are several types of progestins. Synthetic analogues of progesterone are the most commonly used compounds for prevention or suppression of estrus in dogs.[1] They act by blocking the production and/or the release of GnRH from the hypothalamus, most likely by negative feedback. Estrus in bitches is prevented more frequently by depot formulations of progestins (medroxyprogesterone acetate, delmadinone acetate, and proligestone). To suppress signs of estrus without any side effects, it is recommended to treat bitches after the third day after the beginning of proestrus (when parabasals cells are still present in vaginal smear). Oral megesterol acetate (0.05 or 0.01 mg/kg) was effecive for a year with less side effects when treated during anestrus.[2] Later, a higher dose (0.55 mg/kg/day for 32 days) was recommended based on sucessful treatment in anestrus in 98% of anestrus animals (n = 200).[3]

In contrast to past protocols, it is no longer recommended to repeat treatment throughout life.[1] This is due to the concern about potential side effects. Although still controversial, the main concern is the proliferation of mammary parenchyma, potentially leading to the development of benign or malignant mammary gland tumours after repeated treatment. Among others, skin discoloration at the site of injection, anabolic effects, increased appetite with subsequent weight gain, insulin resistance and suppression of the adrenocortical axis are commonly reported side effects. Pyometra may occasionally develop, especially when progestins are used in diestrus under endogenous progesterone secretion. Proligestone is a most recent type of progestin released to the market in Europe, and

it has less progestational activity.1 Its side effects on the uterus may therefore be weaker than older drugs.

Deslorelin subcutaneous implants

GnRH agonists mimic the action of endogenous GnRH. Deslorelin is a super-agonist in which the stability of the molecule and its affinity to the GnRH receptor are increased. It interferes with the pituitary gland via GnRH receptors and thus FSH/LH secretion. In Europe, it is marketed under the form of a slow-release subcutaneous implant (Suprelorin®) that contains either 4.7 or 9.4 mg of deslorelin. The action of deslorelin occurs in 2 phases. First, in the days after implantation, a stimulation phase occurs leading to increased LH and FSH release that may induce signs of estrus. This 'flare-up' effect has a short duration (2 - 4 weeks). It is followed by pituitary inhibition due to a postreceptor mechanism leading to inhibition of the messenger RNA (mRNA) encoding the β-subunits of the gonadotropins.[4] Consequently, plasma concentrations of FSH and LH are at basal concentations and thereby estrus is prevented. However, this effect is temporary and therefore limits the use of deslorelin for control of dog overpopulation (stray dogs). Conversely, deslorelin is an option to be considered as an alternative to surgical spay in pets, because a new implant can be administered at the time of annual vaccination.

Use in adult bitches

Deslorelin has the major disadvantage of inducing estrus in nearly 100% of adult bitches before the desired inhibitory phase.[5] This initial induction of estrus just after implantation limits its use in practice. Several attempts have been made to avoid the consequences of this 'flare-up' effect. To date, none of the protocols is 100% effective.[6] Implantation of deslorelin in bitches during diestrus when progesterone concentrations are high (> 5 ng/ml) was 1 approach. However, estrus was observed in some implanted bitches, even when plasma progesterone concentrations were > 60 ng/ml.[6] Further attempts were made with the concomitant or preimplantation treatment of progestagens, acylin (GnRH antagonist), anastrazole (aromatase inhibitor), clomiphene acetate (antiestrogen) or osaterone acetate (antiandrogen).[6]

Additionaly, deslorelin implants had adverse effects in adult bitches.[7] Signs such as persistent estrus, ovarian cysts, lactation, and behavioural changes were reported.[8] However, the occurrence of these side effects seems rather unpredictable, and only a few individuals were affected. All implanted bitches should be closely monitored, especially during the 'flare-up' period, because the majority of side effects, although uncommon, are observed during that time. Prolonged estrus, ovarian cysts, and pyometra were observed in a bitch implanted with deslorelin after hCG (human chorionic gonadotropin) treatment to induce ovulation.[9] In most cases, removal of the implant solved the problem within 15 days.[8] However, in some cases, ovariohysterectomy had to be performed. In a retrospective study[7], uterine pathologies were observed in bitches with or without clinical signs of postimplantation estrus. In these cases, the treatment of choice would also be surgical removal.

Implantation is recommended during anestrus10 since treatment during diestrus (high progesterone concentrations) increased the risk of uterine diseases (e.g. pyometra). Once the animal is no longer under the effect of the GnRH agonist, cyclicity returned and future fertility was not affected.[11] The duration of estrus prevention was 10.2 ± 5.1 months on average with 4.7 mg implants, but it varied considerably from 2 - 27 months.[6,8] Often, the first natural estrus that follows implantation is norma, and the bitch ovulates normally. For bitches reimplanted several times, suppression of estrous signs was observed up to 4 years.[12] This individual variation of estrus suppression is unpredictable and should be considered a further limitation. Therefore, prior to using this implant in breeding bitches, this possible side effect should be discussed with the owner, especially because it is not possible to predict when the bitch will come back into estrus. Suppression of reproductive cyclicity was successfully achieved in 6 of 10 bitches for 1 - 4 years.[12] No behavioral and local or general side effects were observed in any of the treated bitches. According to these authors, the 4.7 mg deslorelin implant may work well for suppression of cyclicity if implanted in diestrus and reimplanted at intervals of 4.5 months. Deolrelin 9.4 mg implant may be more suitable for this use, although its efficacy may also be shorter than 12 months. According to these authors, owner compliance is an important limiting factor. Our clinical experience is that if the bitch does not develop any side effects in the first weeks after the first implantation, she can be reimplanted regularly several times and even life-long without any problems. We assessed fertility (ovulation rate, pregnancy rate, and litter size) in induced estrues after implantation.[11] Animals were grouped according to treatment responses: group 1 – females exhibiting signs of estrus (n = 19); group 2 – reimplanted (4.7 mg deslorelin acetate) females exhibiting subsequent spontaneous estrus (n = 7); and group 3 – females (4.7 mg deslorelin acetate) evaluated at subsequent spontaneous estrus (n = 13). There were no differences between induced estrus and posttreatment spontaneous estrus in groups 1 and 2 (short-term treatment) nor between spontaneous estrus in group 3 (long-term treatment). Treatment with 4.7 mg deslorelin implant did not compromise the bitches' fertility in subsequent estruses in any group.

Deslorelin implant in older bitches (> 5 years) should be used with caution, as potentially the return to cyclicity may be prolonged (> 27 months) and fertility may be affected due to age. It is recommended that owners sign an informed consent, as this is 'off-label' use.[10] In all cases, it is mandatory before implanting a bitch to examine the ovaries and the uterus by ultrasound (to ensure that no ovarian cysts nor cystic endometrial hyperplasia are present).[10]

Based on our own experience, sedation is usually not necessary to insert the implant under the skin. However, it is recommended to clip and disinfect the skin in order to avoid any local complications. Due to potential side effects, we recommend to implant the bitch subcutaneously posterior to the umbilical area.[5] In case of any further problems, the implant can be easily removed with a small skin incision under slight sedation. Other authors prefer implanting under the skin of the medial part of the rear leg.[13] The matrix of the implant is biocompatible and dissolves very slowly.

It is not necessary to remove the implant and furthermore it is difficult to remove the implant several months after implantation.

Use in prepubertal bitches

The major interest of implanting a prepubertal bitch is to prevent the onset of estrus without inducing estrus. The age at which an animal is implanted is very important, as several studies indicated that all bitches implanted > 6 months of age had signs of induced estrus. Deslorelin implant in 4-month-old females postponed estrus until at least 13 months of age; however, estrus was induced in bitches that received the implant at 7 months of age.[14] Nevertheless, deslorelin implants can be safely used in 4-month-old prepubertal bitches without causing a 'flare-up effect' that commonly occurs in adult anestrous females during the first month after deslorelin implantation.[15] Similar observations were made in another study.[16] Deslorelin implants appeared to be a safe and reliable approach to reversibly postpone puberty in female dogs < 6 months. No estrous signs were observed for 13 - 24 months and 8 - 15 months (observation period) after treatment with a 4.7 or a 9.4 mg deslorelin implant, respectively.[16]

A 4.7 or 9.4 mg deslorelin implant was used in crossbreed prepubertal female dogs (n = 13, 4 - 5.1 months old) as a long-term and reversible contraceptive.[17] Puberty was postponed until 82.7 ± 8.9 weeks in all dogs treated with deslorelin implants (versus 61.9 ± 9.7 weeks in nonimplanted bitches) and epiphyseal closure was delayed, without apparent side effects. Juvenile vaginitis was observed in 75% of the treated females between weeks 10 and 72, but resolved spontaneously.[17] Later, the authors assessed the effects of prepubertal use of deslorelin implants on luteal function after first spontaneous estrus. The long-term delay of puberty by a deslorelin implant in prepubertal female dogs aged 4.2 months appeared to have no negative carry-over effects on subsequent reproductive activity and ovarian functionality.[18] A 4.7 or a 9.4 mg deslorelin implant delayed puberty in 4 month old female dogs and did not cause uterine disturbances. [19] Another study[20] evaluated effects of repeated treatment of a 4.7 mg deslorelin implant in 4.5 month old female dogs. No estrous signs were recorded after 3 consecutive treatments at 4.5 month intervals, demonstrating that puberty was efficiently postponed. Regular closure of bone physes was observed, but hip dysplasia was recorded in 2 deslorelin-treated dogs. Juvenile vaginitis was also reported in 75% of the cases; nonetheless, they recovered within 2 weeks after implantation. Although deslorelin inhibits ovarian activity, a potential consequence is postponement of external genital development and maintenance at an infantile stage.

We investigated the use of deslorelin 4.7 mg (group 1) and 9.4 mg (group 2) subcutaneous implants for the postponement of puberty in bitches < 6 months of age.[21] No bitch in group 1 had any sign of induced estrus soon after implant, and owners recorded no clinical side effects. Bitches had their first estrus between 13 - 24 months after implantation and had an elevated plasma progesterone concentrations (> 15 ng/ml), indicating that ovulation had occured. None of the bitches that underwent puberty had any abnormality of fur, growth or development of external genitalia. No bitch in group 2 had estrous signs until the end of the observation period (8 - 15 months).

Other potential uses of deslorelin subcutaneous implants

GnRH receptors have been identified in several tissues (mammary gland, genital tract, bladder, and hair follicles) and deslorelin subcutaneous implants have been tried in several 'off-label' indications.[22,23]

Medical control of reproduction in the bitch: areas of research

Hormonal manipulation

Aromatase inhibitors have been used for estrus prevention in foxes.[24] There are some unpublished data in the bitch using a drug called finrozole. In an experimental trial conducted at the University of Kuopio in Finland for patent reasons (EP 1399158 A1), using oral finrozole at a dose of 3 mg/dog/day from the first day of estrous signs for 21 days, it was observed that in 2 of 6 dogs, estrogen secretion decreased to the extent that the signs of estrus disappeared. However, in 4 dogs, although estrogen concentrations decreased, estrous signs persisted. The weak response was obviously due to the very low dose used.[24] However, as the fox has reproductive physiology closely resembling dog, aromatase inhibitors may be an option in the near future.[25]

Antiprogesterone drug aglepristone (Alizin®, Virbac) treatment in the preovulatory period of the bitch in our studies did not block ovulation.[26] However, it had a contraceptive effect by delaying oocyte maturation and preventing progression of sperm in the oviduct.

Contraceptive vaccines

Three types of contraceptive vaccines are possible:[27] 'classic' subunit vaccines, containing antigenic fragments of the target; DNA vaccines allowing cells to produce antigens directly to elicit an immune response (the vaccine is thus introducing into certain cells of the organism the gene coding for the vaccine antigen); or recombinant vaccines produced by genetic engineering that use a microbial vector that has been rendered harmless (the genes coding for the vaccine target are introduced into the vector microorganism). AntiGnRH immunization appears ideal, because this hormone is the 'heart' of reproductive function. However, GnRH molecule, a simple decapeptide (10 amino acids) is not very immunogenic. To create a vaccine, it must therefore be conjugated with more antigenic substances. Another approach is to combine the action of a toxin to destroy the target. An antagonist or an antiGnRH antibody conjugated with a toxin, once introduced into the target cell, can destroy it. However, GnRH neurons are difficult to reach because of the blood-brain barrier. In addition to GnRH, immunization trials with FSH or its receptor can lead to sterility by action on the Sertoli cells in males and on the granulosa cells in females. Immunization trials against LH or its receptor have

allowed the bitch to achieve reversible infertility for about a year.[28] Vaccines against local targets[27] (e.g. proteins of the zona pellucida) have several side effects (ovarian cysts, ovarian inflammation, prolonged proestrus or estrus, and abnormal hormone profiles). Currently, contraceptive vaccines are not available in carnivores.[29] There are local reactions related to adjuvants, the duration of efficacy is extremely variable among individuals and their development is made very difficult by ethical concerns and the cost of studies.

Kisspeptins

Canids are particularly sensitive to kisspeptins. Injection of KP-10, a kisspeptin agonist, causes a faster and greater increase in gonadotropins compared to other mammals.[30] Therefore, kisspeptins are interesting therapeutic targets for the suppression of fertility in dogs and cats.

Among several kisspeptin analog molecules developed in recent years, only the kisspeptin agonist canine KP-10 and kisspeptin antagonist p271 and p234 peptides (tested for antagonistic properties on the kisspeptin receptor) were tested in dogs. Although they exhibited antagonistic activity in many mammalian species, these molecules had no substantial effect in the bitch, irrespective of the phase of the estrous cycle,[30,31] suggesting that the canine kisspeptinergic network is species specific, and remains to be studied.

A kisspeptin vaccine, KISSI, injected intramuscularly into lambs 3 times at an interval of 3 weeks, induced a strong antibody response and resulted in the suppression of gonadal function and sexual behaviour,[32] demonstrating that it can be used as a novel target for developing a DNA immunocastration vaccine. It would be effective in domestic carnivores because of adequate conservation of the kisspeptin structure between species. Inhibition of kisspeptin synthesis by silencing RNA (siRNA) is also an interesting method that is being researched.

Kisspeptins are promising therapeutic targets for nonsurgical sterilization of domestic carnivores. Advances in research in experimental rodents, ruminants, and even humans, suggest new uses of these molecules in dogs. However, switching from 1 species to another is not easy and makes studies in target species necessary in order to develop the optimal solution.

Gene silencing

Genetically mediated contraception ('gene silencing') is probably one of the promising approaches for development of new techniques for contraception.[29] The principle is to inhibit the genes that activate certain functions of reproduction. Gene silencing is basically blocking the expression of a particular gene, usually through the use of an interfering RNA (RNAi). The disruption is carried out at the level of the transcription of the gene, or of its protein translation, causing inhibition of gene expression. There is no doubt that this is an extremely vast field of research and currently very promising.

Conflict of interest

None to report.

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