FACULTY OF VETERINARY MEDICINE
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Alopecia X in Pomeranian dogs

Word count: 8132

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A dissertation submitted to Ghent University in partial fulfilment of the requirements for the degree of Master of Veterinary Medicine

Academic year: 2021 – 2022

GHENT UNIVERSITY

Contents

Summary

Alopecia X is a skin disorder that occurs in Pomeranians and other plush-coated breeds. Dogs are presented with bilateral, symmetrical, non-inflammatory hair loss. Multiple names were given to the disorder with black skin disease (BSD) being one of the most known. The exact cause of the disease remains unknown but recent studies have shown that genetic deregulation in sex hormones could be one of the causes of alopecia X. As a consequence of the onset at a fairly young age and the prevalence in the mentioned dog breeds, the disorder is suspected to be hereditary. Therapies differ for every individual patient, as multiple treatments exist. No treatment is perfect for resolving the disorder and multiple treatments can be attempted at the same time. Neutering, melatonin, trilostane, medroxyprogesterone acetate, deslorelin and previously mitotane were used as possible treatments. Recently microneedling and fluorescent light therapy are getting more common in veterinary practice to treat alopecia X. Microscopically alopecia X is visible as hair cycle arrest.

This retrospective study consists of the cases treated by Ghent University's Faculty of Veterinary Medicine throughout the last years. Differences in treatment, amount of hair regrowth and if the dogs relapse into hair loss are set side by side for each individual.

Alopecia X is een aandoening van de huid die voorkomt bij de Pomeriaan en andere rassen met een pluizige vacht. Honden worden gepresenteerd met bilateraal, symmetrisch, niet-inflammatoir haarverlies. Er zijn meerdere benamingen aan de aandoening gegeven, waaronder black skin disease (BSD) een van de bekendste is. De exacte oorzaak van de ziekte blijft onbekend maar recente studies hebben aangetoond dat een genetische ontregeling van de geslachtshormonen een van de oorzaken kan zijn van alopecia X. Als gevolg van het ontstaan op vrij jonge leeftijd en de prevalentie bij de genoemde hondenrassen wordt vermoed dat de aandoening erfelijk is. Therapieën verschillen voor elke individuele patiënt, er bestaan meerdere behandelingen. Geen enkele behandeling lost de aandoening volledig op en er kunnen meerdere behandelingen tegelijk worden ingesteld. Castratie, melatonine, trilostane, medroxyprogesteronacetaat, deslorelin en vroeger mitotane werden als mogelijke behandelingen gebruikt. Recentelijk worden microneedling en fluorescerende lichttherapie meer en meer toegepast in de diergeneeskunde om alopecia X te verhelpen.

De retrospectieve studie bestaat uit de patiënten behandeld door de Faculteit Diergeneeskunde van de Universiteit Gent. Verschillen in behandeling, mate van haargroei en of de honden in hun afwijking hervallen worden voor elk individu naast elkaar gezet.

Introduction

Alopecia X is a skin disorder that occurs in Nordic, plush-coated breeds. Pomeranians, Samoyeds, Chow Chows and Malamutes are often diagnosed with alopecia X and presented with bilateral symmetrical hair loss. In the last decennia, this disease has been named pseudo-Cushing's syndrome, castration-responsive alopecia, biopsy-responsive alopecia, congenital adrenal hyperplasia-like syndrome and more recently hair cycle arrest or alopecia X. Because of the prevalence in the mentioned dog breeds and the onset at a young age the disease is considered to be hereditary. It is a non-inflammatory skin disease in which the hair cycle is disrupted. Telogen hair follicles predominate and anagen follicles are sparse. The exact cause of alopecia X is yet to be discovered.

One of the first hypotheses was growth hormone shortage. When growth hormone was administered to affected dogs, positive effects were visible in some cases. Hair regrew in some dogs with alopecia X but results were very inconsistent and causality was never proven (Cerundolo et al., 2007; Frank et al., 2004; Frank and Watson, 2013). Other theories focus on the deficiency in sex hormone production. Different methods of treatment corroborate the assumption that a disbalance in sex hormones is possible. Hair regrowth after neutering is visible in approximately 60% of the cases (Huang et al., 2009). A study regarding the estrogen receptor in affected dogs showed mild to moderate hair regrowth in some cases following 3 months of oral melatonin. In mice, estrogen receptors are important for regulating the telogen-anagen transition. The hypothesis that dogs who receive melatonin will regrow hair because of interaction with these estrogen receptors remains unproven (Frank et al., 2006). In recent research genome sequencing was used on skin samples of dogs with alopecia X and important genes coding for hormone-regulating enzymes were identified with changes. These changes contain a substantial factor in the upregulation and downregulation of the enzymes which help produce hormones such as vitamin D, sex hormones and melatonin (Brunner et al., 2017). This research enhances the hypothesis that these hormones are altered locally in the skin of alopecia X-affected dogs.

Initially symptoms are prevalent on the bilateral sides of the body. The loss of primary hair will be the first step. A dull, dry coat is visible in the neck as the animal's collar will cause much friction. A rat-like tail is visible and the back of the neck will appear alopecic. Secondary hair will remain for a more extended time. Other than hair loss, the animals affected with alopecia X do not show any signs of illness. If dogs affected by hair loss show other symptoms it is necessary to test for other systemic diseases. Diabetes mellitus, hypothyroidism, hyperestrogenism and hyperadrenocorticism are important differential diagnoses of alopecia X. Skin hyperpigmentation results from sunlight exposure due to lack of hair (Nuttall, 2008).

A definite diagnosis can be made by excluding other possible disorders such as endocrine diseases, which cause the same symptoms as alopecia X (Frank, 2015). Routine laboratory testing is helpful to rule out differential diagnosis of alopecia X, therefore hematology, chemistry panel, urinalysis, thyroid function tests and adrenal function tests can be performed in suspicious cases. After excluding endocrine diseases, a skin biopsy can be performed for histopathology.

Histology of a skin biopsy sample alone will not give enough information in differentiating alopecia X from other metabolic skin diseases that cause the same type of changes. Histopathology findings in alopecia X include orthokeratotic hyperkeratosis, follicular keratosis, dilation of the follicular infundibulum, epidermal melanosis and many hair follicles in the telogen phase (Müntener et al., 2012). Hairs with delayed catagen development show trichilemmal keratinization and flame follicles are present. These signs are typical for most endocrinopathies (Frank et al., 2006). Breed, age and

alopecia distribution must be taken into account. The onset of hair loss happens between 2 and 6 years (Cerundolo et al., 2004).

Treatment of alopecia X is complex, as not every treatment is helpful. Multiple treatments exist but none of them is perfect for resolving the disorder. Since the true etiology is still unknown, it often remains a search for the right treatment for every individual patient.

Neutering is the initial treatment of choice and in most cases hair growth will start within 8 weeks after surgery. Although relapse is possible, partial or total regrowth is visible in most dogs after castration (Gondim and Araujo, 2020). Forty-three percent of male Pomeranians had complete hair regrowth within 6 months after castration. However, partial hair regrowth is seen in 17,1% of dogs within 6 months after surgery (Huang et al., 2009).

Melatonin administration results in hair regrowth for unknown reasons. Melatonin influences hormones such as oestradiol and testosterone as melatonin has a negative effect on GnRH secretion. In goats the supplementation of melatonin resulted in the transition of hair follicles from telogen into the pro-anagen phase (Fischer et al., 2008). The pineal gland produces the neurohormone melatonin in response to the day-night cycle, therefore melatonin controls the circadian rhythm and hair growth cycles. A study during treatment with melatonin showed hair regrowth in 62% of dogs with alopecia X (Frank et al., 2004).

Trilostane is another possible treatment for dogs with alopecia X. Trilostane is a competitive inhibitor of 3β-hydroxysteroid dehydrogenase, the enzyme essential for cortisol and progesterone synthesis. In humans trilostane is used since the 1970s to treat Cushing's syndrome and breast cancer (Cerundolo et al., 2004). Adverse effects of trilostane are unpredictable but mild to moderate and occur in up to 40% of cases. Electrolyte abnormalities, decrease in appetite and vomiting are most common. Trilostane is metabolized in the liver and excreted in bile and urine. Usage is not recommended in dogs with renal insufficiency or liver problems (Lemetayer and Blois, 2018). A study in which 16 Pomeranians and 8 miniature poodles were treated, showed complete hair regrowth in 14 out of 16 Pomeranians. The other 2 Pomeranians showed no hair regrowth. Hair regrowth took place within 4 to 8 weeks in most Pomeranians and miniature poodles (Cerundolo et al., 2004).

Hair regrowth appears locally in areas where trauma or biopsy took place. This indicates that small trauma is beneficial for hair growth. The practice of microneedling, with tiny needles puncturing the skin, has been used in human medicine since the early 2000s. The creation of microchannels induces minor skin injury with minimal damage, this stimulates the dermal wound healing. Inflammation, proliferation and remodelling occur and growth factors are released locally (Alster and Graham, 2018). Skin trauma seems to initiate the anagen phase of the hair (Stenn and Paus, 2001; Stoll et al., 2015). Anaesthesia or deep sedation is needed to be able to do this procedure, despite this inconvenience the treatment is simple without any severe side effects. Hair regrowth is expected within 12 weeks after the first treatment (Stoll et al., 2015).

Mitotane is a pharmaceutical product that was used in the treatment of hyperadrenocorticism (PDH). It inhibits enzymes near the adrenal cortex and has a cytotoxic effect on adrenal cortex cells (Bikas et al., 2019). In dogs tested with mitotane treatment after treatment with melatonin, partial to complete hair regrowth was seen in more than half of the dogs (Frank et al., 2004). In a study with 200 dogs on mitotane, 50 showed weakness, vomiting, anorexia, diarrhoea and ataxia as side effects (Kintzer and Peterson, 1991). Mitotane was sold as Lysodren and is not available anymore in Belgium.

Medroxyprogesterone acetate results in partial or complete hair regrowth in half of the dogs tested. In the study, existing out of 2 groups, 8 dogs were tested. In the first group of the study 4 out of 5 dogs

showed some hair regrowth. In the second group of the study 1 out of 3 dogs showed minimal hair regrowth. Averagely the same results were obtained with melatonin (Frank et al., 2004). Progesterone will induce mammary derived growth hormone which helps dogs in growth hormone deficiency. In Pomeranian dogs insulin-like growth factor remains low during treatment, unlike in normal-coated dogs. No adverse reactions occurred (Frank and Watson, 2013).

Implanting these alopecic dogs with deslorelin via subcutaneous implant is another possibility. The reaction of the hair follicle on this pharmaceutical is not yet understood. The overall response to therapy was about 60% in recent studies. Deslorelin inhibits GnRH production, adverse reactions are follicular cysts and prolonged estrus (Albanese et al., 2014).

A new technology in veterinary medicine is the use of FLE (fluorescent light energy) which has beneficial effects in skin adaptation to wound healing and hair regrowth. Tissue proliferation takes place by influencing angiogenesis and collagen production (Apostolopoulos and Mayer, 2020).

Diagnostics work up in a Pomeranian with suspicion of alopecia X

Hair cycle arrest, also known as alopecia X, occurs in male and female dogs. It is important to form a signalment of the dog, sex and castration are both important factors of the anamnesis. Neutering is currently one of the first choices of therapy in male dogs. Other factors like breed and age are important. More often alopecia X occurs in intact male animals between 1 and 3 years of age (Cerundolo et al., 2004; Frank, 2015; Nuttall, 2008). Clinical signs can be progressive, bilateral and symmetrical. The only loss of primary hair is visible with secondary hair remaining present in most cases. Most commonly primary hair is scarce in places with much friction. A rat-like tail is visible, dorsal-caudal on the back, caudal on the thighs and the perineal region are primary places of hair loss. Hyperpigmented skin is visible, which is why the disease is also called black skin disease. The distal aspect of the paws remains normal. These factors are important for differential diagnosing the disorder at first glance (Frank et al., 2004; Gross, 2005; Rothstein, 2011).

Another aspect of alopecia X is the health of the affected animal. The dogs with this disorder should be healthy animals in every other aspect, the disorder is merely a cosmetic defect. Routine haematology and biochemistry in blood testing should show normal results (Cerundolo et al., 2004).

Clinical differential diagnoses should include other pathologies which influence the endocrine system. Hypothyroidism, hyperadrenocorticism, Sertoli cell cancer-associated skin disease and follicular dysplasia are common disorders that can show similar clinical symptoms as alopecia X. Even diabetes mellitus can cause symptoms comparable to alopecia X, it is advised to check for these conditions first. Endocrinologic function tests, serum biochemical tests, history, clinical development of the alopecia, and histopathology may be valuable in differentiating among these diseases (Gross, 2005).

Abdominal ultrasound imaging can be used to exclude hyperadrenocorticism. It is a widely used diagnostic imaging technique whereby bilateral or unilateral adrenomegaly and hyperadrenocorticism can be diagnosed. In Pomeranians the thickness of the adrenal gland is expected to be a maximum of 5.1 mm for dogs up to 5 kg (Melián et al., 2021).

As stated before, histopathology can be one of the diagnostic tools to link a patient to suffering from alopecia X. It is still not the ideal identification tool as it does not differentiate alopecia X from other non-inflammatory skin disorders. Superficial skin layers appear to be normal in these patients, with epithelium being of normal thickness and follicular epithelium being unaffected. Hyperpigmentation is visible in the epidermis due to the accumulation of pigment after sun exposure. Hair follicles are in a state of arrest, they retain the hair shafts and as a result staying in the telogen phase (Gross, 2005). In biopsy samples flame follicles are noticeable and prominent. Flame follicles typically occur in skin with increased trichilemmal keratin. Trichilemmal keratin appears as a bright amorphous matter which looks like a flame. In the anagen phase of the hair cycle, this type of keratin is scarce but the amounts will rise during the catagen phase. Trichilemmal keratin in a brush-like pattern will appear at the base of the hair follicle while spreading deeper into the root sheath and the vitreous layer. When fully replacing this root sheath the telogen phase starts (Gross, 2005; Ordeix et al., 2002). In endocrine follicular disorders, dysplasia of the follicles can be seen sporadically (Rothstein et al., 1998).

When taking biopsies, it is important for all cases of endocrine disease to pick the right biopsy location. Taking skin from the most affected regions is necessary to identify the problems going on in the skin. Skin from areas with hair growth should be avoided and multiple samples must be obtained.

Novel insights

Recent research conducted by Brunner et al. in 2017, have shown new insights into regulation of the skin affected by alopecia X. To perform this study skin biopsies were taken from Pomeranians affected by alopecia X and healthy control Pomeranians. RNA was extracted from these skin samples. If the RNA reads passed the quality control, they were mapped to a genome reference. The expressed genes were then mapped to a biological network, this showed 569 genes were upregulated and 1029 genes were downregulated in dogs suffering from the skin disorder.

Genes of the Wnt, Shh, Bmp, Fgf and Tgf-β were analyzed as they are known to play a major role in the hair follicles homeostasis (Lee and Tumbar, 2012). Forty-seven genes which were participating in the hair pathways were selected. Expression of stem cell markers were found to be upregulated or downregulated.

The pathway cycle shows the physiological function of differentially expressed genes in dogs affected by alopecia X (Figure 1). Different genes are needed to start different advancements in the hair cycle.

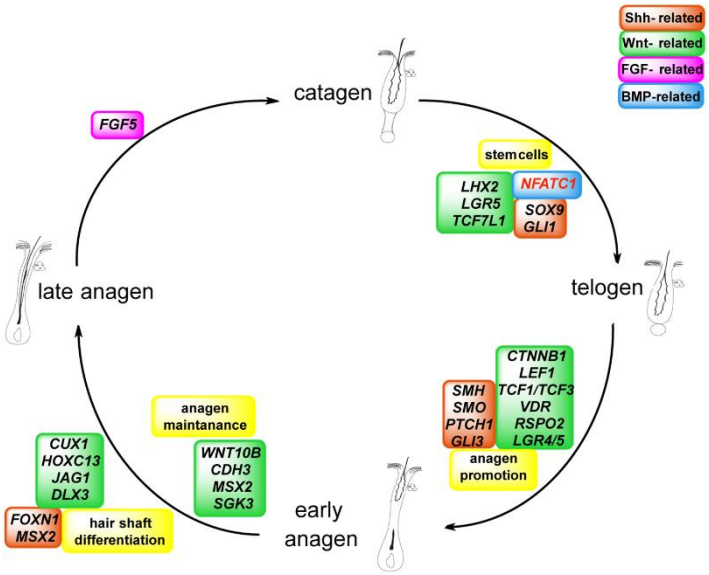

Figure 1: affected pathways and genes in dogs with Alopecia X (Brunner et al., 2017)

The dataset which was used to derive genes from skin biopsies also showed underlying deregulation of genes which are important for steroid hormone metabolism. This has been one of the major theorized causes of alopecia X in recent years (Cerundolo et al., 2007; Frank et al., 2006). Sex hormone dysregulation is hypothesized to occur on a different level in the skin with the skin having its own neuro-endocrine system, with functions comparable to hypothalamus-hypophysis-adrenal axis, catecholaminergic, cholinergic and steroidogenic systems. (Slominski and Wortsman, 2000).

CYP1A1 and CYP1B1 were found to be upregulated, these genes play a factor in the metabolism of oestrone and oestradiol. CYP1A1 and CYP1B1 also affect the shift of normal melatonin to 6-OH-melatonin, this causes a change in the melatonin/6-OH-melatonin offset which affects gonadotropin releasing hormone. KISS1 and WNT5A were downregulated and play a role in the metabolism of the gonadotropin releasing hormone metabolism and thus dysregulation the hypothalamus-hypophysis-adrenal axis. HSD17B14 is also downregulated and plays a factor in androstenediol, testosterone, esterone and oestradiol metabolism by coding for the enzyme 17β-hydroxysteroid dehydrogenase. The estrogen receptor ESR2 is also downregulated (Brunner et al., 2017).

Possible treatments

Multiple treatments are possible for treating hair cycle arrest (alopecia X) in Pomeranians but with the exact cause of the skin disorder remaining unknown it can be difficult to set up the right treatment from the start. It is a difficult search for the right treatment in every individual patient, some dogs have more side effects to some therapies than others and some therapies are better to be avoided in some patients.

Neutering

When hair cycle arrest of unknown origin occurs in intact dogs, neutering can be the first treatment of choice. Mostly male, intact dogs are presented with alopecia X. For some time after surgery, from a couple of weeks to months to years, hair regrowth may occur. In most male dogs the surgical treatment shows partial or complete hair regrowth within 3 to 4 months (Cerundolo et al., 2004). Because a re-occurrence of hair growth is visible in some cases, alopecia X can be named castration responsive alopecia. Patel & Forsythe (2010) state that neutering should result in hair regrowth in both male and female Pomeranians. In approximately 15% of the cases relapse of alopecia is possible (Gondim and Araujo, 2020).

A study done by Huang et al. (2009) in 141 Pomeranians, of which 35 male Pomeranians were affected with hair cycle arrest, showed 42,9% of hair regrowth within 6 months. These dogs did not show any relapse of alopecia X in the following 3 to 10 years. Partial hair regrowth was visible in 17,1% of the dogs. In these dogs hair regrowth came within the first 6 months after castration. In the remaining dogs showing hair cycle arrest, the treatment didn't show any improvements and alopecia increased steadily.

Recent genetic sequencing found changes in a couple of genes important for hormone metabolism in dogs with hair cycle arrest. CYP1A1 and CYP1B1 were found to be upregulated, these genes play a factor in the metabolism of oestrone and oestradiol. KISS1 and WNT5A were downregulated and play a role in the metabolism of the gonadotropin releasing hormone metabolism and thus dysregulation the hypothalamus-hypophysis-adrenal axis. The synthesis of certain sex hormones was affected. For this study, skin samples were taken from several Pomeranians suffering from alopecia X. Melatonin and vitamin D metabolism was also affected. This may explain the connection with the influence of the sex hormones on alopecia X (Brunner et al., 2017).

Melatonin

Hair follicles are a target for multiple hormones and other transmitting molecules. The hair follicle is both a target and source of prolactin, oestrogen, cortisol and corticotropin-releasing hormone, with melatonin being a proven neuroendocrine mediator (Fischer et al., 2008). As the skin seems to be reacting to melatonin in approximately 40% of the dogs with hair cycle arrest, if administered over a period of 3 months (Frank et al., 2006), a link to melatonin dependency can be expected. In another study done by Frank et al. (2004) approximately 62% of the dogs regained partial or complete hair regrowth.

Melatonin is produced by the pineal gland, following the circadian rhythm, and influences multiple other reproductive systems. It is proven that melatonin treatment can significantly decrease oestradiol, testosterone, 17-hydroxyprogesterone and dehydroepiandrosterone sulphate levels in

intact dogs (Ashley et al., 1999). So it is hypothesized that melatonin can normalize sex hormone concentrations in intact dogs affected by alopecia X.

Mammalian skin has been shown to express full enzymatic functions to produce melatonin, this was proven in situ in mice and humans (Kobayashi et al., 2005). Melatonin stimulates the hair follicle to develop from the resting telogen phase into the pro-anagen phase, which results in increased fur development in mammals (Paterson and Foldes, 1994).

The recommended dose of melatonin is 3 mg BID orally for small breeds and 6–12 mg BID orally for large breeds (Frank et al. 2004). Melatonin should be avoided in dogs with diabetes mellitus because a risk of insulin resistance in high doses (Espino, 2011).

Trilostane

Trilostane functions as a steroidogenesis inhibitor, through its effect on 3β-hydroxysteroid dehydrogenase. It is used in the treatment of Cushing's syndrome and breast cancer in humans. As a result of the interaction with this enzyme, pregnenolone, 17-hydroxypregnenolone, dehydroepiandrosterone and androstenediol are not conversed into their effective hormonal successors. Production of all classes of steroid hormones, including androgens, oestrogens, progestogens, glucocorticoids and mineralocorticoids is blocked (de Gier et al., 2011). Thus, suggesting a link with sex hormone metabolism, treatment has the same purpose as neutering.

Complete hair regrowth can be expected in 85% of Pomeranians, although in some Pomeranians no regrowth is visible. If hair regrowth is visible, it should be noticeable withing 4 to 8 weeks but longer periods may be possible. In this study with a higher dose (11,76 mg/kg on average) than prescribed (2 mg/kg) effect rates remain the same (Cerundolo et al., 2004). When hair fully regrew a maintenance dose was established three times per week. Trilostane can reduce the synthesis of testosterone and has an anti-progesterone effect (de Gier et al., 2011), it should always be avoided in pregnant bitches.

Treatment with trilostane should be done with Modrenal or Vetoryl. A starting dose of 2 mg/kg is recommended, orally with food. An ACTH-stimulation test should ideally be performed before starting the treatment, with check-ups after 10 days, 4 weeks, 12 weeks and 3 months being recommended (directions given with Vetoryl 60mg). Check-ups during the trilostane-treatment should show a sufficient decrease in cortisol (de Gier et al., 2011; Galac et al., 2010), side effects should be rare or absent. When side effects occur, they are unpredictable but mild to moderate in most cases. Electrolyte abnormalities, decrease in appetite and vomiting are the most common. Trilostane is metabolized in the liver and excreted in bile and urine. Dogs who suffer from renal disease or liver insufficiency should never be treated with trilostane (Lemetayer and Blois, 2018).

Microneedling

Microneedling has been used to improve hair regrowth in human medicine for many years. It is used for aesthetic purposes and has been studied in acne scars, melasma, androgenic alopecia and skin rejuvenation (Alessa and Bloom, 2020). When looking at areas with trauma or areas of which biopsy took place, hair regrowth can be visible in these spots. By creating multiple traumatic microchannels it is possible to copy this effect, these microchannels can be made with a Dermaroller®. Dermarollers are widely available drum shaped devices with protruding needles and have been used by humans for hair growth stimulation for decades.

A study done by Kim et al. (2016) showed hair growth promotion in mice who were treated with a disk microneedle roller. During the study the expression of Wnt3a, β-catenin, vascular endothelial growth factor (VEGF) and Wnt10b were increased. Wnt-signalling molecules maintain the hair activity in dermal papillae (Kishimoto et al., 2000), β-catenin will act as a transducer in the Wnt-signalling pathway. β-catenin will translocate to the nucleus and stimulate transcription of the vital genes. VEGF will stimulate capillary growth through angiogenesis in the skin, therefore transportation of necessary nutrients to the hair follicles.

Minimal epidermal damage and the controllability of skin injury are important positive features of microneedling. When used on scar tissue, the microneedles will tear compact collagen strands and will induce the production of new elastin and collagen. The establishment of these microscopical wounds promote the release of growth factors which directly play a role in dermal cell activation (Alster and Graham, 2018). Growth factors such as platelet-derived growth factor, which plays a role in angiogenesis, fibroblast growth factor and transforming growth factor alpha and beta will help in re-establishing healthier skin.

Anaesthesia of the dogs is necessary for each treatment, this can cause the owner to be averse against the treatment. Before the treatment, dogs should be bathed with an antiseptic shampoo. The treatment consists of rolling the Dermaroller® over the alopecic areas in 3 directions, diagonally, vertically and horizontally. Each direction should be rolled 4 to 5 times during each session. Capillary bleeding is taken as the endpoint of treatment. After microneedling, the skin should be disinfected with a 2% chlorhexidine spray. Needle lengths of 1.5 mm to 2.5 mm showed improvements in hair regrowth (Stoll et al., 2015).

When using microneedling in a Pomeranian, scaling of the skin can be visible within 2 weeks post-treatment. Within 5 weeks diffuse hair growth should be visible and after 12 weeks approximately 90% of hair regrowth should be established (Stoll et al., 2015).

Mitotane

o,p'DDD or mitotane was sold as Lysodren. It has been used in the treatment of adrenocortical carcinoma and cushing's syndrome and is derived from the insecticide DDT. Mitotane is a cytolytic drug which affects the zona fasciculata and the zona reticularis of the adrenal cortex. In dogs the zona glomerulosa can be affected in about 6-10% of the cases, aldosterone secretion can be affected (Hart and Reagan, 1973).

Mitotane has its effect on the mitochondria, which have an important role in cancerous tissue and apoptosis of the cells. Mitotane induces weakening of the respiratory chain, following aspartate to decrease and glutamate to increase (Ségolène Hescot et al., 2017). Furthermore, mitotane interferes with the 11β-hydroxylase enzyme (CYP11B1), this causes in decreased steroid synthesis. The cytotoxic mechanism should also result in a decrease of cortisol precursors. Frank et al. (2004) state that androstenedione, progesterone and 17-OHP are suppressed similarly to cortisol, but oestradiol remains unaffected which results in uncomplete suppression of hormones in some dogs. They expect that the hormone which influences hair regrowth was not measured or all of the effects of mitotane on hair growth are not hormone dependent.

In dogs tested with mitotane treatment after treatment with melatonin, partial to complete hair regrowth was seen in more than half of the dogs (Frank et al., 2004). Mitotane was also used in dogs with pituitary dependent hyperadrenocorticism (PDH) with a dosage of 21 to 69 mg/kg for 5 to 14 days. A lot of adverse side effects during the treatment appeared, some of the dogs exhibited 1 or more side effects. Weakness, vomiting, anorexia, diarrhoea and ataxia were common in 25% of the initial 200 dogs. 35% of the dogs had low post-ACTH serum cortisol concentrations, which means the mitotane treatment had a positive effect. 25 dogs still reacted to ACTH and produced high cortisol levels, therefore achieving no effect of the mitotane treatment (Kintzer and Peterson, 1991).

Lysodren was produced by Bristol Myers Squibb and is not available anymore in Belgium.

Medroxyprogesterone acetate

Medroxyprogesterone acetate (MPA) is used as a heat cycle suppressor in female dogs and is sold as Provera®. The usage of a progestogen like medroxyprogesterone acetate does bring some risks, occurrence of reproductive and non-reproductive disorders are possible. Bilateral ovarian cysts, cystic endometrial hyperplasia, pyometra, mammary adenoma, fibrosarcoma and cystic-papillary adenocarcinoma are identified after long term use of MPA (Keskin et al., 2009).

A study performed by Frank and Watson (2013) showed medroxyprogesterone acetate to result in partial or complete hair regrowth in 50 percent of the dogs tested. In the study, existing out of 2 groups, 8 dogs were tested. In the first part of the study 4 out of 5 dogs showed some hair regrowth. In the second part of the study 1 out of 3 dogs showed minimal hair regrowth. On average the same results were obtained with melatonin (Frank et al., 2004).

Progesterone will induce mammary derived growth hormone which helps dogs in growth hormone deficiency. In Pomeranian dogs insulin-like growth factor remains low during treatment, unlike in normal-coated dogs. No adverse reactions occurred (Frank and Watson, 2013)

Monthly injections with medroxyprogesterone acetate can be a possible treatment in dogs. Treatment is done by subcutaneously injecting the medroxyprogesterone acetate, which provides a slow release and long duration of effect. The starting dose can be set on 5 mg/kg for every 4 weeks for a total of 4 injections. After the first 4 months a new injection dose can be started if the desired effect was not reached, 10 mg/kg is advised (Frank and Watson, 2013).

Deslorelin implant

The hypothesis that deslorelin could be used as a valuable treatment was tested and adopted. Deslorelin acts as an agonist of gonadotropin releasing hormone, thus following the hypothesis of a sex hormone dependant alopecia. It has an effect on luteinizing hormone and follicle-stimulating hormone by their downregulation of the pituary gland. Suplerolin®, produced by Virbac is the commercial name and is available in a 4,7 mg implant which is sterile and can be planted subcutaneously. Deslorelin is a safe and reversable way of sterilising male dogs and remains effective during a period 6 months.

The reaction of the hair follicle on this pharmaceutical is not yet understood. In recent studies the overall response to this therapy was about 60%, when tested in 20 dogs. The dogs in the study were followed for 1 year and there was a possibility that after the follow up and the decreasing effectiveness of deslorelin, a relapse of hair cycle arrest and alopecia would have happened. In the study no female dogs showed any regrowth of hairs. Twelve out of the sixteen intact male dogs in this study showed an increase in hair growth following this deslorelin implant (Albanese et al., 2014).

Follicular cysts, prolonged oestrus and pyometra have been reported in bitches after receiving a 4.7 mg deslorelin implant to prevent oestrus (Ponglowhapan, n.d.). Prior to deslorelin implantation in bitches a good clinical exam must be performed, possible pregnancy or reproductive disorders should be ruled out. Regular health checks are important to detect early side effects (Brändli SP et al., 2021).

Klox's FLE-therapy

Klox technologies is a company which is specialized in skin care in humans and animals. Recently they started working together with Vetoquinol to develop and commercialize Klox's fluorescent light energy (FLE) products in animal health. FLE-therapy by Vetoquinol is called Phovia®.

Fluorescent light energy uses polychromatic light energy to penetrate into multiple layers of the skin. The therapy consists of 2 important elements: the blue light-emitting lamp and the chromophore gel. This chromophore gel needs to be mixed by using a colourant and a gelatinous substance. After mixing the gel, it can be applied evenly on the affected skin. The lamp can then be used to light the gel on the skin, which will cause the chromophores to react and release a fluorescent light into the skin. The lamp's cone should be held as close as possible to the skin without the lamp touching the gel, after a cycle the lamp will stop its beam automatically. It is a quick and non-painful procedure which can be done weekly without sedation being required.

Promotion of a skin healing process can be one of the aims when using Phovia®. In a study done in a dog with skin infection caused by canine calcinosis cutis, the dogs showed big improvements during treatment, the general skin quality improved noticeably. The patient had increased hair growth in the treated areas. After 7 weeks of therapy the treatment was stopped after noticing marked cytological improvements (Apostolopoulos and Mayer, 2020).

Inflammatory mediators are modulated, but unnecessary in non-inflammatory skin diseases like hair cycle arrest. Tissue proliferation takes place and angiogenesis is improved by different growth factors (such as TGF-ß, FGF, PDGF and VEGF) (Apostolopoulos and Mayer, 2020; Marchegiani et al., 2020). Collagen deposition is enhanced thus enhancing the skin healing, comparable to microneedling.

Retrospective research

The retrospective study found results for neutering, melatonin, trilostane, microneedling medroxyprogesterone and deslorelin. Nineteen dogs were found in the clinic's history, of which 12 were male and 7 were female. All of the dogs were Pomeranians. Therapy, dose, hair regrowth and time interval were set side by side.

Neutering

Neutering showed mild hair regrowth in two dogs, of which one of the dogs received more skin stimulation thanks to microdermabrasion under anaesthesia. In the female dog no improvements were noticed in the first 7 months after castration.

Both of the male dogs who received castration resulted in mild hair regrowth. In dog number 2 hair regrowth stopped shortly after the procedure, resulting in a relapse of hair loss. The dogs were later administered melatonin to try and support the hair regrowth after neutering.

Neutering	*Patient*	*Sex*	*Hair regrowth*	*Time interval*	*Relapse*
	1	M	**Mild**	6 weeks	
	2	M	**Mild**	< 1 year	Yes
	3	F	**No**	7 months	

Table 1: effects of neutering

Melatonin

Melatonin was the most used therapy in these dogs. Thirteen dogs were put on this therapy to support other therapies. Nine male dogs and four female dogs were administered melatonin. Nine out of the thirteen dogs received a melatonin implant. Both implant and oral medication showed improvements in some dogs. Of the male intact dogs, one dog became untraceable and a follow-up was never conducted. Of the other four male intact dogs, only two showed improvements with melatonin. The progression was visible within the year. The first dog was also administered deslorelin but relapsed in the disease of hair loss quickly. Of the three male castrated dogs which received melatonin therapy per oral, all dogs showed mild to complete hair regrowth. Two of these dogs relapsed in their disorder shortly after stopping the melatonin therapy. The female dog who was implanted with melatonin gained moderate hair regrowth. The owner of this female dog explained that hair regrew on the initial spots of hair loss, while other spots on the hind legs showed progressive alopecia. These evolutions occurred at the same time after 3 months went by without any changes. The female neutered dog showed no improvements with both implants.

Melatonin	*Patient*	*Sex*	*Dose*	*Hair regrowth*	*Time interval (months)*	*Relapse*
	1*	M	Implant	Mild: focal	3	Yes
	2	M	Implant	No	3	
	3	M	Implant	Unknown		
	4*	M	Implant	No	8	
	5	M	3 mg po	Mild: diffuse	1,5	
	6	MC	3 mg po	Mild: diffuse	12	Yes
	7	MC	3 mg po	Complete	2	Yes
	8*	MC	Implant	No	3	
	9	MC	3 mg po	Mild: focal	3	
	10	F	Implant	Moderate	3	Other locations
	11*	FC	Implant	No	3	
	12*	FC	Implant	Complete	12	Yes, focally
	13	FC	Implant	No	3	

Table 2: effects of treatment with melatonin, oral medication or implants

**: Dogs have been treated with deslorelin and a melatonin implant.*

Trilostane

One dog was treated with trilostane. The dog only received Vetoryl for 2 months and the medication was stopped after no positive effects were noticed. The owner quickly wanted to stop the medication because risk of side effects. Due to high-risk factors most owners did not start treatment with Vetoryl.

Vetoryl	*Patient*	*Sex*	*Hair regrowth*	*Time interval*
	1	MC	-	2 months

Table 3: effects of treatment with trilostane

Microneedling

Only 1 dog was treated with microneedling, this therapy showed large improvements in a female neutered dog. Within 3 months after the procedure complete hair regrowth was visible. Eleven months after the procedure she relapsed into hair loss. She was treated with melatonine and deslorelin before, which didn't stimulate any hair regrowth.

Microneedling	*Patient*	*Sex*	*Hair regrowth*	*Time interval*	*Relapse*
	1	FC	Complete	3 months	Yes

Table 4: effects of microneedling

Medroxyprogesteron acetate

Another therapy of which only one dog was registered is medroxyprogesterone acetate. Only one female dog was treated with this therapy without any results within the first month. No further follow-up took place. The dog was not treated before with any other of the described possible therapies.

Medroxyprogesterone acetate	*Patient*	*Sex*	*Hair regrowth*	*Time interval*
	1	FC	No	1 month

Table 5: effects of treatment with medroxyprogesterone acetate

Deslorelin

Deslorelin is one of the other more frequently used therapies in the university's clinic. The implant is often placed at the same time as a melatonin implant to improve hair regrowth at a higher level. Of the nine dogs treated, two dogs were not traceable after receiving the implant. One of the female neutered patients was treated with both deslorelin and melatonin before, but only showed hair regrowth after microneedling in a later period of time. Of the male dogs only two showed any progression within the first year. The male neutered dogs showed no improvements. Of the female neutered dogs one dog showed complete hair regrowth within a year but relapse occurred focally.

Deslorelin	*Patient*	*Sex*	*Hair regrowth*	*Interval*
	1	M	Mild: focal	7 months
	2*	M	Mild: focal	3 months
	3	M	Unknown	
	4*	M	No	8 months
	5	MC	No	
	6*	MC	No	3 months
	7	MC	Unknown	
	8*	FC	No	3 months
	9*	FC	Complete	12 months

Table 6: effects of treatment with a deslorelin implant

**: Dogs have been treated with deslorelin and a melatonin implant.*

Conclusion of the retrospective research

The best therapy for each patient remains a difficult search even when performed by experienced dermatologists in the university's clinic. When looking at one of the best described therapies, which is neutering, hair regrowth can be seen in both male dogs. Hair regrowth started within 6 weeks after surgery, this is supported in literature (Cerundolo et al., 2004; Huang et al., 2009). In one of the male dogs showing mild regrowth, relapse occurred quickly within a year. This was described by Gondim and Araujo (2020) as being a possibility of 15%.

Melatonin is often administered orally, four dogs who received oral medication regrew hair. Of these four dogs only two relapsed after stopping therapy. When being administered through an implant is becomes more difficult to perform regular check-ups on the patients. Although it is much easier for the owners to get an implant for their dogs, it is more difficult for the university clinic to check if the medication has any long-term improvements. In total seven out of twelve dogs who have been checked up on, had mild to complete hair regrowth. Two of these dogs relapsed. This is supported by the study done by Frank (2004) in which 62% of the dogs regrew hair during a melatonin treatment.

Trilostane was only suggested to three dog owners, only one dog was treated with the medication. When suggesting this possibility the owners were always aware of the possible risks of using a cytotoxic therapy even though in literature no extreme side effects were noticed when administering a low dose (Lemetayer and Blois, 2018). Frank and Watson (2013) researched the success rate of trilostane in Pomeranian dogs, which resulted in a success rate of approximately 50%. This cannot be compared to this retrospective research as only one dog received this medication for a short time.

In five dogs melatonin was placed together with a deslorelin implant. The effect of each individual therapy remains unknown in these patients. In two of the patients no further follow-up was possible. Three of the nine dogs who received a deslorelin implant had hair regrowth, one of these three dogs had complete hair regrowth withing 1 year after implantation. It is likely that the hair growth in this female neutered dog can be caused by a mixture of both deslorelin and melatonin therapies. In three out of five dogs which received both implants no hair growth was noticeable. In a study performed by Albanese et al. (2014) 75% of male intact dogs had hair regrowth, this contrasts the findings in this retrospective research in which only 50% of the male intact dogs showed mild hair regrowth. This difference can be caused by a difference in the number of dogs researched, it is possible that in a study with more dogs higher percentages of success can be attained. In the study done by Albanese et al. (2014) hair growth was absent in all of the female dogs, which were all neutered. In the retrospective research one of the neutered female dogs showed complete hair regrowth.

Other therapies were only tested in one dog each. Microneedling had the best effect on the affected dog but relapse occurred rather quickly. In these single cases it is impossible to form a comparison to the studies performed by others.

Microneedling can be one of the therapies researched in the future as no literature can be found to describe the success rates in a larger population of dogs. It is a relatively new technique used in dogs affected by alopecia X. It has only been described in siblings (Stoll et al., 2015). Klox's FLE-therapy is relatively new in veterinary medicine as well and can also be researched so success rates can be monitored in the future.

Discussion

Alopecia X is a disorder which is very difficult to treat because of the unknown reasons that the hair follicle remains in a state of arrest. Only testing of therapies by trial and error can be done to solve this disorder. If a veterinarian wants to treat alopecia X, multiple therapies can be advised to the owners of the dogs. The first option should always be to check if the dog is neutered or intact. If intact, neutering can be the first choice of treatment, even though it appears that melatonin can have beneficial effects on hair growth by decreasing oestradiol, testosterone, 17-hydroxyprogesterone and dehydroepiandrosterone sulphate levels in intact dogs (Ashley et al., 1999). The combination of melatonin and deslorelin can be safely used in healthy male and female neutered dogs. The owners can choose between a melatonin implant or tablets. For both melatonin and deslorelin implants the dog should be under anaesthesia. The anaesthetic procedure can be combined with microneedling. If the dog accepts treatment with Phovia® FLE light therapy, this can be combined with melatonin and deslorelin as well. Some therapies should be avoided in certain dogs, such as the usage of medroxyprogesterone acetate which can cause reproductive and non-reproductive disorders (Keskin et al., 2009). Also treatment with melatonin should be avoided in dogs with diabetes mellitus because overdosage can cause a risk of insulin resistance (Espino, 2011). Dogs who suffer from renal disease or liver insufficiency should never be treated with trilostane as it should be metabolised in both organs (Lemetayer and Blois, 2018). In intact female dogs reproductive disorders are reported when implanted with deslorelin (Ponglowhapan, n.d.). Therapies have to be based on clinical effects and success rates without compromising the health of the patients

Most of the therapies used in today's veterinary medicine can be easily done by a first line veterinarian. The problem lies within the diagnostics of the disorder. The veterinarian requires to have knowledge of the disorder which is predisposed in certain dog breeds (Frank et al., 2003). The symptoms should be linked to the breed of the dog, which can lead the veterinarian in a specific direction to form a differential diagnosis. Further diagnostics can be more difficult for the average first line veterinarian as it includes abdominal ultrasound of the adrenal gland (Melián et al., 2021) and histopathology of specific skin regions (Gross, 2005). If the veterinarian is able to take useable biopsies it can be advised to send them to a specialized veterinary laboratory for identification and diagnostics. If uncertain a knowledgeable veterinarian should be contacted or referral to a specialized veterinarian dermatologist is advised.

It is very important to remind the owners of the dog that the disorder is purely a condition that affects the cosmetic aspect of the dog. The dogs affected by alopecia X are supposed to be clinically healthy dogs without any underlying illnesses. The fact that a lot of dogs relapse in hair loss also has to be mentioned to the owners.

One of the possibilities to eradicate the disease, can be genetic matching of parent dogs. A hereditary factor of the disorder is theorized (Mausberg et al., 2007). If any research would be performed in the future, a genetic reading could be performed to acknowledge if any hereditable factor plays a major role. If so, breeding lines could be traced and excluded from future reproduction.

Even if any therapy works and stimulates hair regrowth, it is a possibility that new hair growth may not be permanent. Hair regrowth might remain normal for a short period of months to years. Relapse in hair loss might leave owners displeased with the treatment. The observations were also made when contacting owners about the therapy provided during this retrospective research. In this study ten dog owners were contacted personally through phone calls. The others were looked up in the university clinic's database. Most of the owners with dogs who suffered relapse remained unsatisfied and lost hope for any hair regrowth to ever occur. Most owners of these dogs noticed that it happened during

the seasonal change of the fur. Some of the owners are still hoping for a future medical discovery so their dog can be helped.

Some therapies can be preferred over others, based on the signalment of the dogs. In the retrospective research both male dogs, who were neutered as the therapy of choice, had hair regrowth. The female dog did not show any improvements. This therapy is better to perform in male dogs as it is less invasive. In literature neutering is stated to be the treatment of choice in male intact dogs (Cerundolo et al., 2004). Allegedly melatonin could be used as the first treatment of choice in male intact dogs as it is safe to administer in most cases of alopecia X. Melatonin decreases oestradiol, testosterone, 17-hydroxyprogesterone and dehydroepiandrosterone sulphate levels in these intact dogs (Ashley et al., 1999). The same conclusion can be made by comparing the effects of deslorelin on male and female dogs in the retrospective research. The male intact dogs gained more effect from the implant than the neutered male dogs. No female intact dogs were put on deslorelin, results showed hair regrowth in only one of the neutered female dogs. Research performed by Albanese (2014) shows that 75% of male intact dogs treated with deslorelin had hair regrowth. In the same research four female neutered dogs were treated without success. In a study done by Frank and Watson (2013) medroxyprogesterone acetate helps hair regrowth in three out of eight dogs, of which five were female. All dogs in this study were neutered or spayed and only one out of the three male dogs had hair regrowth. The retrospective research done for this paper only shows one dog on medroxyprogesterone acetate, which did not have any hair regrowth. Hair regrowth was only checked after 1 month, the ideal duration of therapy takes at least 16 weeks. It is suspected that the success rates of the other treatments talked about in this paper should not be affected by sex and signalment. Future findings may contradict this statement.

Recently a dysregulation was discovered on the level of the skin, which caused the up- or downregulation of important enzymes of the stem cells in the skin. This dysregulation is theorized to play a major factor in the skin disorder causing alopecia X. Further research should be performed by comparing datasets from affected dogs to datasets from dogs with other conditions with similar symptoms. If any difference can be found, a possible cause for alopecia X could be discovered. This is also hypothesised by Brunner. Older established theories that estrogen metabolism is differentially regulated in the skin of dogs with alopecia X are supported by this recent finding (Brunner et al., 2017).

In summary it can be said that a lot of details remain unknown about alopecia X. The recent studies which show the skin completely regulating differently, form a good lead which should be further investigated. If any conclusions can be made from further research and investigation of this lead, a focus can be made on a specific therapy. If in the future a focus can be made on one specific therapy to help all dogs with alopecia X, the therapy can be adapted to the specific patient to form an ideal situation in which the veterinarian can stimulate the dogs hair growth. Until then the therapies talked about in this paper should be used.

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