

DOI 10.24425/pjvs.2022.142023

Original article

Research on the effect of an androgenic hormone product on the functional status of common sebaceous glands in dogs

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Abstract

Nowadays, a high incidence of cutaneous tumors is observed in domestic carnivorous in pet clinic, the sebaceous glands being the starting point for the development of these tumors. The hormonal imbalances are considered to be the most common etiology for these tumors, so the current research is based on the effects of an androgenic like hormonal drug on the functionality of the sebaceous glands in dogs. For this purpose, 32 dogs were distributed in 4 groups: control group 1 (8 dogs - females), control group 2 (8 dogs - males), experimental group 1 (8 dogs - females) and experimental group 2 (8 dogs - males).

The investigation targeted the pH of skin and the rate of sebum's secretion. The animals from the experimental groups were treated with Anabolin forte for 2 days at a dose of 1 mg/kg body weight/day, intramuscular.

The obtained results revealed that administration of Anabolin forte in males induced a significantly distinct intensification (with 10.66%) of sebum's secretion and a significant decrease of pH of the skin (with 17.1%) compared to the animals from the control group. The administration of Anabolin forte in females induced a significantly distinct intensification (with 17.47%) of sebum's secretion and a significant decrease of the pH of the skin (with 14.32%) compared to the animals from the control group.

Key words: sebaceous glands, sebum, pH, androgenic hormone

Introduction

Skin tumors are a very common neoplasms affecting dogs. Incidence rates are around 450 to 100,000 dogs (Vail et al. 2001). The most frequent skin tumors in dogs are ones benign. The histiocytomas and sebaceous gland adenomas are the most frequent (Goldschmidt et al. 2002).

The sebaceous glands of domestic carnivores can represent an anatomical site for the development of skin tumors, which can be diagnosed on the entire surface of the skin. However, skin tumors occur mainly on the head, abdomen and chest (Özyiğit et al. 2005, Toniti et al. 2009).

The exact etiology of skin tumors in domestic carnivores is not known, but recent research indicates an involvement of a hormonal imbalance, which appears to play the decisive role. Sex steroid hormones also appear to be involved in this imbalance (Rosser 1992, 2000, Brunetti et al. 2013). Preclinical studies have shown that castration of male dogs with skin tumors has led to a partial clinical remission of tumor syndrome (Zielonka et al. 1994, Burrai et al. 2010). Other studies have also identified a possible relationship between sex steroid hormones and sebaceous and perianal tumors (Martins et al. 1997, Hernblad et al. 2008, Mouser et al. 2010).

Estrogen, progesterone and androgen regulate skin development. Also, the development and function of sebaceous glands are regulated by these hormones (Kariya et al. 2005). In this context, recent biochemical and immuno-histochemical studies have identified androgen, estrogen and progesterone receptors in the epidermis and in the sebaceous glands in dogs (Kariya et al. 2005, Ginel et al. 2006, 2010, Frank et al. 2006, Brakta-Robia et al. 2009). These results suggest that androgens, estrogen and progesterone, may play roles in the normal skin functions and in the pathogenesis of neoplasms.

Studies show that canine sebaceous glands express high levels of estrogen receptor α (ER α) and also, progesterone receptors (PR). Estrogen receptor α expression was significantly lower in sebaceous gland epitheliomas and adenocarcinomas. These data suggest that estrogen receptor α plays a major role in the physiology of sebaceous glands and a decrease of these receptors influences the development of skin tumors. (Scott et al. 2000, Ginel et al. 2010) This drop in estrogen receptor α levels could be secondary to changes in androgen or estrogen production (Hirsch et al. 1993, Blackwood 2011).

However, hormonal receptors are present in the basal cells of normal sebaceous glands in dogs, there is little data regarding the involvement of steroid hormones in the pathology of skin tumors.

Sex steroid hormones seem to be involved in the development of animal skin tumors. Current research in this field shows the potential benefits of endocrine manipulations of tumor progression.

This increasingly obvious hormonal etiology has led to a deepening of research on the effect of sex hormones, and especially androgenic hormones, on the structure and functions of the sebaceous and perianal glands in dogs and cats (Hamilton and Montagna 1950, Haris et al 1992, Sobczak et al. 2002, McGinley et al. 2007).

The present paper is aimed to elucidate the effect of androgenic hormonal therapy on the functional status of the sebaceous glands in dogs.

Materials and Methods

Thirty two mixed-breed dogs were used in the experiment (16 females and 16 males), with estimated ages between 3 and 5 years.

The animals stayed inside the paddocks of the Biobase of the Faculty of Veterinary Medicine Bucharest, during the experiment, where all the legal standards for housing and treatment of laboratory animals were strictly respected.

All methods used in this experiment were performed in accordance with relevant guidelines and regulations.

Our study was carried out in accordance with the ARRIVE guidelines.

The experimental animals had granulated food and water *ad libitum*, during the experiment. The experiment was approved by the ethics commission of the Faculty of Veterinary Medicine Bucharest. The experiment did not affect the health or the individual comfort of the dogs in the experimental groups.

1. 32 dogs were grouped into 4 experimental groups;
2. Control Group 1 (n=8) consisting of females;
3. Control Group 2 (n=8) consisting of males;
4. Experimental Group 1 (n=8) consisting of females;
5. Experimental Group 2 (n=8) consisting of males.

The used animals were clinically healthy. In addition to clinical examination, the animals were also evaluated by laboratory tests, by investigating the hematological and biochemical blood parameters, which proved to be within physiological limits.

In order to perform our experiment, we obtained the consent of the owners of the animals used in the study.

The animals from all the experimental groups were evaluated on the first day of the experiment for skin pH level and sebum secretion intensity. These parameters were measured using two devices:

Table 1. Protocol followed during the experiment.

Day	Group 1 (Control group 1-Females) (n=8)	Group 2 (Control group 2- Males) (n=8)	Group 3 (Experimental group 1-Females) (n=8)	Group 4 (Experimental group 2-Males) (n=8)
Day 1	Evaluation of sebum and pH	Evaluation of sebum and pH	Evaluation of sebum and pH	Evaluation of sebum and pH
	-	-	Anabolin forte 1 mg/kg/day	Anabolin forte 1 mg/kg/day
Day 2	-	-	Anabolin forte 1 mg/kg/day	Anabolin forte 1 mg/kg/day
Day 3	-	-	-	-
Day 10	Evaluation of sebum and pH	Evaluation of sebum and pH	Evaluation of sebum and pH	Evaluation of sebum and pH

- Sebumeter - SM 815;
- Digital pH-meter Skin-pH-Meter PH 900.

The animals from groups 3 and 4 were treated with the product Anabolin forte, on the same day. This product was injected for 2 days at a dose of 1 mg/kg body weight/day, administered intramuscularly.

Then, on the 10th day of the experiment, all the animals from the experimental groups were evaluated again: skin pH level and sebum secretion intensity (Table 1)

Anabolin forte contains 19-nortestosterone also known as Nandrolone. This is an anabolic androgenic steroid. Nandrolone is present in the form of nandrolone decanoate in Anabolin forte, being presented under this trade name by Alfasan Diergeneesmiddelen (Netherlands).

The sebumeter is a device designed to measure sebum on the surface of the skin and hair. It is used in dermatology and cosmetics. Various “*in vitro*” and “*in vivo*” tests, as well as comparisons with other measurement techniques, mentioned in the literature, have demonstrated the effectiveness of this method.

The operation principle of the Sebumeter SM815 is based on the photometry of grease stains (sebum), impregnated on a special tape. As the amount of fat (sebum), impregnated in that tape, increases, it becomes more and more transparent.

To determine the sebum, the measuring head of the cassette is inserted into the opening of the device, where the transparency is measured by a light source, which emits light rays through the crossbar and which is then reflected by a mirror placed behind the tape. A photocell measures the intensity of light that manages to cross the tape (transparency). Light transmission indicates the sebum content on the surface of the skin. A micro-processor calculates the result, which is displayed on a screen and expressed in micrograms/cm².

Direct skin measurements involved:

- choosing the surface for making the determinations;

- preparing the surface for performing the determinations.

Scott et al. showed that the skin from the lumbar area is the most suitable for making the above measurements. A square surface was delimited, with an area of 100 cm² (a square with a side of 10/10 cm), placed on the left side of the lumbar area.

The preparation of the area consisted in cutting and shaving the hair, on the surface mentioned above. This was done 24 hours before the start of the experiment.

The order of the measurements was the following: determination of sebum production and then measurement of pH.

Statistical analysis involved calculating the mean, standard error and standard deviation of the mean, using Microsoft Excel. The comparison between the series of values and the statistical relevance was assessed using the t-Student test (Microsoft Excel).

Our experiment took place in the biobase of the Faculty of Veterinary Medicine in Bucharest, benefiting from the favorable approval from the Ethics Commission of this institution and fulfilled the conditions required by EU Directive 63/2010.

Results

The sebum secretion, recorded on the first day of the experiment, shows higher levels in males than in females. These differences were the following:

- in the case of the comparison between groups 1 and 2, the difference was 1.125 µg/cm² (45.3%);
- in the case of the comparison between groups 3 and 4, the difference was 1.005 µg/cm² (38.8%).

In both cases the differences were distinctly significant ($p < 0.005$) from a statistical point of view.

It should be noted that there were no significant differences regarding this parameter, between the males

Table 2. The average intensity of sebum secretion in the case of all experimental groups of dogs $\mu\text{g}/\text{cm}^2$.

The level of sebum secretion	Group 1 (Control group 1-Females) (n=8) Mean \pm SD	Group 2 (Control group 2- Males) (n=8) Mean \pm SD	Group 3 (Experimental group 1-Females+AnabolinF) (n=8) Mean \pm SD	Group 4 (Experimental group 2-Males+AnabolinF) (n=8) Mean \pm SD
Day 1	2.4825 \pm 0.056	3.6075* \pm 0.05	2.5841 \pm 0.033	3.5899* \pm 0.025
Day 10	2.5814 \pm 0.042	3.5162 \pm 0.052	2.9164* \pm 0.041	3.9921* \pm 0.028

* $p < 0.005$

Table 3. The average value of the skin pH in the case of all experimental groups of dogs.

The level of sebum secretion	Group 1 (Control group 1-Females) (n=8)	Group 2 (Control group 2- Males) (n=8)	Group 3 (Experimental group 1- Females+Anaboli F) (n=8)	Group 4 (Experimental group 2-Males+AnabolinF) (n=8)
Day 1	7.065	6.874	7.008	6.822
Day 10	7.122	6.799	6.1025*	5.635*

* $p < 0.05$

from groups 2 and 4, and the females from groups 1 and 3.

The sebum secretion recorded on the 10th day of the experiment was higher in the case of males treated with Anabolin forte, compared to the males from the control group, with the differences of 0.3846 $\mu\text{g}/\text{cm}^2$ and, respectively, 10.66%. This difference proved to be distinctly significant ($p < 0.005$).

The sebum secretion recorded on day 10th of the experiment was higher in females treated with Anabolin forte, compared to females from the control group. The difference between the level of sebum secretion in females treated with Anabolin forte and in females from the control group was 0.4339 g/cm^2 and, respectively, 17.47%. This difference proved to be distinctly significant ($p < 0.005$) from a statistical point of view (Table 2).

The pH of the skin recorded on the first day of the experiment shows lower levels in males than in females. These differences were as following:

- in the case of the comparison between groups 1 and 2, the difference was 0.191 (2.7%);
- in the case of the comparison between groups 3 and 4, the difference was 0.186 (2.65%).

The differences were distinctly significant ($p < 0.005$) in both cases from a statistical point of view.

It should be noted that there were no significant differences regarding this parameter between the males from groups 2 and 4 and the females from groups 1 and 3.

Skin pH in males treated with Anabolin forte was recorded on the 10th day of the experiment and it was lower compared to males from the control group. The difference between the skin pH level in males treated with Anabolin forte and that recorded in the case of males from the control group was 1.164 and 17.1%

respectively. The difference is statistically significant ($p < 0.05$).

Skin pH in females treated with Anabolin forte was recorded on the 10th day of the experiment and it was lower compared to females from the control group. The difference between the skin pH level in females treated with Anabolin forte and that recorded in the case of females in the control group is 1.02 and 14.32%. The difference is statistically significant ($p < 0.05$) (Table 3).

Discussion

Our results indicate significant differences in the level of sebum secretion between the females and males in dogs. The explanation is that this parameter is amplified mainly by androgenic hormones (testosterone and dehydroepiandrosterone), while estrogens exert reducing effects (Özyiğit et al. 2005, McGinley et al. 2007, Hernblad et al. 2008, Ginel et al. 2009). The differences between sexes, regarding the pH level of the skin, are certainly caused by the increased level of fatty acids in the epidermis, as a result of an increased secretion of sebum (Diani et al. 1992, Liang and Liao 1992, Brunetti et al. 2013).

The results obtained by us are in accordance with those obtained by others (Ebling et al. 1967, Pincus 1987, Ginel et al. 2009) who found higher levels of sebum secretion in adult males compared to adult females in humans, rats and guinea pigs, compared to prepubescent animals.

Anabolin induces an obvious amplification of sebum secretion through its specific androgenic effects. This effect is obvious in the case of females treated with

this drug, these conclusions being also confirmed by the results presented by some other authors (Ebling et al. 1967, McGinley et al. 2007, Blackwood 2011).

Our results regarding the pH level of the skin in the animals from the experimental groups are in direct correlation with those obtained by us in the case of evaluating the level of sebum secretion. Lowering the pH of the skin is a natural consequence of the high content of fatty acids in sebum. It is obvious that Anabolin forte induces a decrease in the pH of the skin, through its specific androgenic effects. This effect is obvious in the case of females treated with this drug.

Conclusions

Our results indicate significant differences in the level of sebum secretion between females and males, these levels being higher in males than in females.

Also, the pH level of the skin is lower in the case of males than in the case of females, the differences being statistically significant.

The product Anabolin, based on 19-nortestosterone, also known as Nandrolone, had a stimulating effect on the sebum secretion, on both females and males.

Also, Anabolin had a significant diminishing effect on the sebum secretion, in both females and males.

Our result showed that male sexual steroid hormones can modulate the functions of sebaceous glands in dogs.

These hormones seem to be involved in the etiology of animal skin tumors.

Our results suggest that further research in this field shows potential benefits in endocrine manipulations of tumor progression.

Acknowledgements

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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