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WHITE NEW ZEALAND RABBIT INTRAORBITAL GLANDS – AGE, WEIGHT AND MORPHOMETRY

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Abstract

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The deep gland of the third eyelid (Harderian gland), the inferior and superior lachrymal glands is basic structural and functional units in the rabbit intraorbital gland·s complex. The lack of data about the weight and morphometrical agest parameters of the rabbit intraorbital glands in the specialized literature motivated us to conduct the following investigation. In the present study is used material from the Harderian gland, the inferior and superior lachrymal glands of 48 white New Zealand rabbits divided equally from both sexes into 8 groups – 21, 28, 36, 43, 51, 58, 94 and 110 days old. After the removal of the glands before fixation, were determined their weight, longitude and circumference of every organ. The results were calculated with statistical computer program. The analysis of the retrieved data proved that during the examined period the weight of the Harderian gland of the New Zealand rabbits increased 13.25 times, the longitude increased 3.13 times and the circumference increased 2.37 times. The weight of the inferior and superior lachrymal glands increased 10.8 and 4.51 times, the longitude increased 2.9 and 3.01 times, and circumference increased 1.83 and 3.15 times.

Key words: Rabbit, Intraorbital glands, weight, morphometry

Introduction

Micheluzzi (1967) conducts the first complex description including identification, anatomical and topographical peculiarities, blood supply and inervation of the glands forming the intraorbital glandular complex in the domestic rabbit. According to this study in the eye orbit of the domestic rabbit are situated the following anatomically differentiated glands: *gl. lacrimalis superior*, *gl. lacrimalis acessoria*, *gl. lacrimalis inferior*, *gl. palpebrae tertiae profunda* (*gl.* Harderiana) and *gl. palpebrae tertiae superficialis*. Of her researches who conducted their studies later confirms the description of Micheluzzi (1967), but denies the thesis for the presence of glandula lacrimalis accessoria in the orbit of the domestic rabbit (Bjorkman et al., 1970; Kuhnel, 1971 and etc.). Nowadays according to Sakai (1999) is accepted the opinion that in the rabbit *gl. lacrimalis accessoria* lacks.

After a series of scientific evidences, nowadays exists a uniform opinion, that the gland of the third eyelid (Harderian gland) in the birds is a basic determining factor of the level of the local immunity in the ocular orbit. After the publication of Schlegel et al. (2003) demonstrating the significance of the third eyelid and its associated glands in several species of domestic mammals, for the level of Ig A and secretors components in the lachrymal secretion increased significantly the interest towards the mammalian Harderian gland, which made it a desired research object. The histochemical and ultrastructural peculiarities in norm and after a number of different influences is comparatively well studied mainly over rodents used basically for experimental and laboratory purposes – rats, mice, gerbils, guinea pigs, rabbits and etc.

The number of studies in the specialized literature describing the weight and morphometrical parameters of the Harderian gland or any of the other mammalian intraorbital glands is extremely scarce. Only Their (1999) determines the dimensions of the nuclei in the secretory cells of glandula intraorbitalis in the white rats, and Lopez et al. (2003) conducts an ultrastructural morphometrical study of the myoepithelial cells in the gerbils Harderian gland. In the rabbit, the weight and morphometrical investigations are mainly over

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organs from the digestive system (Jackowiak et al., 1996; Zamborsky – Kovach et al., 2002), and one study of Dragin et al. (2006), in which is made a comparative morphometry between the mammary glands of transgene and non-transgene rabbits. In three scientific articles – Christiansen et al. (1996), Shimokawa et al. (2002) and Gultiken, et al. (2006), is presented data for morphometrical studies of the eye orbit and extra orbital space of the New Zealand rabbit, but their object of research is the muscular apparatus of the eye or the periorbit. Only Meneray et al. (2001), concisely and without explanations for which of the lachrymal glands is their study informs that the sensory denervation of the rabbit's lachrymal gland, do not leads to a change in its structural and morphometrical parameters.

According to Grigorov, et al. (1992), the New Zealand rabbit because of its meat bred use, its rapid maturity and fertility is one of the most popular breeds bred in Bulgaria during the best several years. We did not found in the specialized literature data for age or morphometrical parameters of the rabbit's intraorbital glands. This fact, the opportunity to use biological material from 8 age groups of rabbits from this breed, as well as previously determined weight and morphometric indices in New Zealand rabbit's Harderian gland (Dimitrov et al., 2007), motivated us to conduct the present study. In it, we aimed ourselves to determine the weight and morphometrical parameters of the basic glands forming the intraorbital glandular complex of the New Zealand white rabbit during the first seven weeks after the weaning of the nursing rabbits.

Materials and Methods

For the conduction of the present study was used biological material from 48 New Zealand white rabbits – 21, 28, 36, 43, 51, 58 and 110 days old. Each of the age groups consisted of 6 clinically healthy rabbits (equally divided from both genders). After inhalation with ether followed by a cervical dislocation, from each rabbit were obtained the left and right Harderian gland (96 from all the 48 rabbits), the superior lachrymal gland (96 numbers) and the inferior lachrymal gland (total 96 numbers), with the use of stereomicroscope TECHNIVAL –2 (Carlzeiss, Jena, Germany). The total count of the obtained intraorbital glands was 288. All procedures were performed as per the requirements of the Trakia University Animal Ethics Committee. For each of the obtained glands after the removal of the surrounding tissues and vessels and before placing in fixating mixture, was determined its weight with an electronic scale "SARTORIUS" AY303 (USA) with precision of 0.001 g. With the use of bands of plotting paper, ruler and caliper, were measured for each obtained gland its length, with and circumference according to the rules of Avtandilov (1990). The results were processed with statistical software (Stat Most for Windows) and presented in tabular form.

After the determination of the weight and morphometrical parameters, the glands were fixated in fixative solution – 100 $g.kg^{-1}$ neutral formalin, and the Bouin's and Carnoy's fixating mixtures. After processing the fixated material following conventional histological methods, from paraffin histological sections after Ehrlich's hematoxylin-eosin staining, a considerable count of durable histological specimens was prepared (Vitanov et al., 1995; Kiernan, 2008). The observation of the prepared specimens with a light microscope "ERGAVAL" (Carlzeiss, Jena, Germany), confirmed the authenticity of the obtained glands.

Results

The conducted ***weight analysis*** determined that the increase of the weight for the completely monitored period of the rabbit Harderian gland is with 1688.83 mg, for *gl. lacrimalis inferior* with 997.50 mg, and for *gl. lacrimalis superior* with 116.667 mg. An increase in weight with approximately similar week values during the completely monitored seven weeks period was not determined for any of the three studied glands. Fore every of the three glands this process was carried out in an even way (Tables 1, 2 and 3).

For the Harderian gland the study determined most significant weight increase – during the second week (28^{-th} – 36^{-th} day) with 306.66 mg, during the week before the last one (58^{-th} – 94^{-th} day with 797.00 mg and at the end of the studied period (94^{-th} –110^{-th} day) with 499.66 mg. Lowest value of the Harderian gland weight increase was determined during the period 43^{-th} – 51^{-th} day – with Just 6.66 mg. For the whole period (21^{-st} – 110^{-th} day) the Harderian gland of the white New Zealand rabbit increased its weight 13.25 times.

Glandula lacrimalis inferior similar to the *gl. palpebrae tertiae profunda* (*gl.* Harderiana) also shows an uneven weight increase – with clearly pronounced growth between 28 – 36^{-th} day with 132.500 mg, in the end of period 58^{-th} – 94^{-th} day with 644.333 mg and between 94^{-th} – 110^{-th} with 162.500 mg. Lowest values of weight growth the inferior lachrymal gland had between 21^{-th} – 28^{-th} day with only 1.667 mg. For the whole studied period 21^{-th} – 110^{-th} day *gl. lacrimalis inferior* increased its weight 10.8 times.

Glandula lacrimalis superior similarly to the Harderian and the inferior lachrymal glands unevenly increased its weight – with a clearly pronounced growth between 43^{-rd} and 51^{-st} day with 42.333 mg and between 58^{-th} and 94^{-th} day with 45.166 mg. Most weakly - with only 2.500 mg the weight of the superior lachrymal gland increase during the period 36^{-th}

Table 1
Weight and morphometrical parameters of *Harderian gland* of New Zealand rabbits (mean ± SEM)

Age, days	Weight, mg	Length, mm	Width, mm	Circumference, mm
21	137.834 ± 2.45	9.667 ± 0.42	6.000 ± 0.37	14.333 ± 0.76
28	160.833 ± 4.73*	14.500 ±0.56**	6.833 ± 0.40	15.333 ± 0.76
35	477.500 ± 3.82**	17.500 ± 0.43**	10.167 ± 0.17**	24.333 ± 0.49**
36	467.500 ± 9.38**	16.667 ± 0.84**	9.833 ± 0.31**	22.833 ± 0.87**
43	480.833 ± 15.41**	19.000 ± 0.63**	9.000 ± 0.26**	22.667 ± 1.36**
51	487.500 ± 18.96**	20.667 ± 1.02	9.667 ± 0.61**	25.667 ± 1.45**
58	530.000 ± 12.29**	24.000 ± 0.82**	11.333 ± 0.33**	30.667 ± 0.92**
94	1327.000 ± 0.03**	27.833 ± 0.31**	12.833 ± 0.31**	33.000 ± 0.56**
110	1826.667 ± 1.22**	30.333 ± 0.33**	13.833 ± 0.31**	34.000 ± 1.03**

* Statistically significant difference compared to 21-st day at $p < 0.05$, determined with Mann-Withney U-test;
** Statistically significant difference compared to 21-st day at $p < 0.001$, determined with Mann-Withney U-test.

Table 2
Weight and morphometrical parameters of *inferior lachrymal gland* of New Zealand rabbits (mean±SEM)

Age, days	Weight, mg	Length, mm	Width, mm	Circumference, mm
21	100.833 ± 4,36	14.666 ± 0.67	4.000 ± 0.26	14.000 ± 1.00
28	102.500 ± 8,83	21.500 ± 1.34**	4.667 ± 0.21	12.167 ± 0.48
35	223.333 ± 5,11**	23.333 ± 0.61**	6.500 ± 0.43**	14.000 ± 0.58
36	235.000 ± 21,56**	22.500 ± 0.67**	6.667 ± 0.42**	13.333 ± 0.21
43	251.667 ± 2,11**	22.833 ± 0.17**	6,833 ± 0,31**	16.167 ± 0.60
51	284.000 ± 5,03**	26.167 ± 0.70**	7,000 ± 0.00**	15.667 ± 0.21
58	291.500 ± 4,96**	27.667 ± 0.61**	7.333 ± 0.21**	17.667 ± 0.56*
94	935.833 ± 26,91**	38.833 ± 1.64**	8.000 ± 0.37**	18.000 ± 0.56*
110	1098.333 ± 0.41**	42.833 ± 0.65**	13.500 ± 0.56**	21.833 ± 0.60**

* Statistically significant difference compared to 21-st day at $p < 0.05$, determined with Mann-Withney U-test;
** Statistically significant difference compared to 21-st day at $p < 0.001$, determined with Mann-Withney U-test.

Table 3
Weight and morphometrical parameters of superior lachrymal gland of New Zealand rabbits (mean±SEM)

Age, days	Weight, mg	Length, mm	Width, mm	Circumference, mm
21	33.333 ± 1.67	6.333 ± 0.33	2,666 ± 0,21	6.167 ± 0.40
28	46.667 ± 1.67**	9.333 ± 0.33**	3,500 ± 0.42	8.333 ± 0.61*
35	47.167 ± 1.30**	9.667 ± 0.21**	4.167 ± 0.31*	8.833 ± 0.60*
36	52.500 ± 3.35**	11.333 ± 1.20**	4.333 ± 0.42**	9.333 ± 0.61*
43	50.000 ± 1.29**	10.667 ± 0.42**	2.833 ± 0.17	9.500 ± 0.50
51	94.833 ± 1.22**	13.000 ± 0.37**	6.167 ± 0.17**	14.500 ± 0.34**
58	98.167 ± 3.42**	13.833 ± 0.70**	6.833 ± 0.40**	15.500 ± 1.09**
94	143.333 ± 3.57**	18.000 ± 0.52**	7.500 ± 0.22**	18.000 ± 0.37**
110	150.000 ± 1.83**	19.167 ± 0.48**	8.500 ± 0.22**	19.500 ± 0.50**

* Statistically significant difference compared to 21-st day at $p < 0.05$, determined with Mann-Withney U-test;
** Statistically significant difference compared to 21-st day at $p < 0.001$, determined with Mann-Withney U-test.

– 43-th day, as for the whole studied period 21-st – 110-th day the weight increased with 4.51 times.

The ***morphometrical studies*** determined, that rabbit's Harderian gland for the period from the 21-st to the 110-th day increased its ***length*** with a total of 20.66 mm, the inferior lachrymal gland with 28.16 mm, and the superior lachrymal gland with 12.83 mm. For the three of the glands it was determined, that the length growth during the completely studied period runs unevenly. The most intensive growth of the Harderian gland is in the beginning of the period – 21-st- 28-th day with 4.83 mm, for the inferior lachrymal gland this was the period 58-th – 94-th day with 11.5 mm, and for the superior lachrymal gland this was also the same period from 58-th to the 94-th day. The most in significant length growth of the Harderian gland was between the 43-rd and the 51-st day with only 1.66 mm. For the inferior lachrymal gland, this was the period between the 36-th and the 43-rd day with a length growth of only 0.33 mm and for the superior one – the period between the 36-th and the 43-rd day with a length increase of only 0.66 mm. For the period from the 21-st to the 110-th day the New Zealand rabbit Harderian gland increases its length 3.13 times, the inferior lachrymal gland 2.9 times, and the superior lachrymal gland 3.01 times.

The ***width*** of the deep gland of the third eyelid of the New Zealand white rabbit for 89 days after the rabbits weaning increased with 7.83 mm, of the inferior lachrymal gland with 9.50 mm, and the superior lachrymal gland with 5.83 mm. The weakest growth process for the Harderian gland is between 21-st – 28-th day with only 0.83 mm, for the inferior gland between the 43-rd and 51-st day, and for the superior lachrymal gland between the 36-th and 43-rd day with a negative growth of 1.5 mm. The width growth process of the Harderian gland was well expressed between the 28-th and the 36-th day with the 3 mm, of the inferior gland was at the end of the period 94-th – 110-th day with 5.5 mm, and for the superior lachrymal gland between 43-rd – 51-st day with 3.33 mm. For the period between twenty first and the one hundred and tenth day, the three intraorbital glands increased their width as follows – the Harderian one with 2.30 times, the inferior one with 3.37 times and the superior one with 3.19 times.

The ***circumference*** of the Harderian gland for the completely studied period increased with 19.66 mm, of *gl. lacrimalis inferior* with 9.66 mm and of *gl. lacrimalis superior* with 13.33 mm. The conducted study determined that as the previously mentioned parameters, the circumference of the three glands increases unevenly during the studied period. The weakest growth of the Harderian gland was determined during the period between the 36-th and the 43-rd day when the circumference did not increased at all. For the inferior gland, this was the period between the 58-th and the 94-thday, when the growth amounted to only 0.33 mm, while for the superior lachrymal gland this period was situated between the 36-th and the 43-rd day with one increase of the circumference with 0.20 mm. For the time of the study (21 – 110 day) the Harderian gland increased its circumference with 2.37 times, the inferior gland with 1.83 times, while the superior lachrymal gland increased its circumference 3.15 times.

Discussion

As was previously mentioned in the preface part, there is a lack of data in the specialized literature for the weight and morphometrical parameters of any of the glands forming the intraorbital glandular complex of the rabbit, which deprives us from the opportunity to compare the results of our study.

The cited in the text and presented in Tables 1, 2 and 3 results from our study gives us a season to state, that for every one of the three studied intraorbital glands, every one of the monitored parameters changes unevenly during the interval of the studied period. A clearly expressed rise of the values of almost all of the studied parameters is noted during the second (28-th – 36-th day) and the last two weeks (58-th – 94-th and 94-th – 110-th day) of the studied period. These is almost no dependency between the growth dynamics and the growth end in the intraorbital glands of the New Zealand rabbit from its weaning (21-st day) until the 110-th day from its postnatal development.

Conclusion

Nevertheless, that this is the first scientific research presenting information about the weight and morphometric parameters of the intraorbital glands of the white New Zealand rabbit, considering the acquired data it can be made several conclusions. It can be said that the intraorbital glandular complex of the white New Zealand rabbit consists of three anatomically differentiated and with a consistent localization glands – a deep gland of the third eyelid (Harderian), inferior lachrymal gland and superior lachrymal gland. Every one of the three glands has a separate and independent of the other two glands development cycle.

As at the day of the weaning (21-st day) of the white New Zealand rabbits, and at the final of the studied period (110-th day), the Harderian gland possesses the highest values of the studied parameters. The acquired results allows us to assert that in the eye orbit of the white New Zealand rabbit the dominating in weight and dimensions is the Harderian gland, followed by the inferior lachrymal gland, and the smallest of the three glands is the superior lachrymal gland.

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