The Effect of Xylene and Formaldehyde Inhalation on Testicular Tissue in Rats*

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ABSTRACT: In this study, changes in testicular tissues of rats subjected to xylene and formaldehyde inhalation were evaluated. Three experimental groups were included in the study. Each group of rats was exposed to formaldehyde (6 ppm), technical xylene (300 ppm) or a combination of these two agents (150 ppm+3 ppm) for 8 weeks (8 h/d). Control groups were maintained for a period of eight weeks under the same conditions. Staining methods (triple staining, strep ABC method) were applied to examine histometric changes and relaxin like factor (RLF) expression in the testicular tissue. Immunostaining for RLF showed that density of staining for RLF decreased in rats exposed to formaldehyde. Formaldehyde or a combination of formaldehyde and xylene led to a decrease in seminiferous epithelial height. In conclusion, exposure of rats to formaldehyde and xylene-formaldehyde combinations adversely affects Leydig cells (RLF) and seminiferous epithelium of testicular tissue. (Key Words : Testes, Relaxin-like Factor, Xylene, Formaldehyde, Rat)

INTRODUCTION

Formaldehyde is a chemical which is traditionally used for fixing cadavers and routine histology and histopathology techniques. In histology, xylene is used for clearing the tissues following dehydration in preparation for paraffin blocking (Golalipour et al., 2007). Environmental pollutants such as xylene, formaldehyde, ethane dimethane sulfonates (EDS), thinner, toluene, methanol have a negative effect on the function and structure of the testis tissue (Steinberger, 1992; Handagama and Ariyaratne, 2001; Karimov et al., 2003).

In studies conducted on rats, xylene and formaldehyde caused testicular atrophy and decreases in testes weight and serum testosterone level, diameter of seminiferous tubules and seminiferous epithelial height (Chung et al., 1999; Lemasters et al., 1999; Ozen et al., 2005; Golalipour et al., 2007).

Relaxin-like factor (RLF), also known as the Leydig cell insulin-like factor (Ley-IL), is a circulating hormone that is synthesized in the mammalian gonads and released into the bloodstream (Bullesbach and Schwabe, 1999; Ivell and Bathgate, 2002). RLF, which is the product of the insulin-like factor 3 (INSL3) gene, is a novel family member of the insulin-like hormone. RLF was detected as protein at a high level in the mature and foetal testicular Leydig cells of males (Ivell and Bathgate, 2002; Bogatcheva et al., 2003). No immunohistochemical staining for Insl3 protein was observed in Leydig cells of rats on postnatal days 1-5. In the foetal rodent, Insl3 is responsible for the first trans-abdominal phase of testicular descent. Insl3 is regulated indirectly through perturbation of foetal Leydig cell differentiation status. In the adult rodent, Insl3 may have an anti-apoptotic effect on male germ cells (Sadeghian et al., 2005).

There have been a limited number of investigations carried out on the effects of xylene and formaldehyde inhalation in testes tissues obtained from rats of different age groups (Karimov et al., 2003; Zhou et al., 2006). No reports on variations in the composition of RLF produced from Leydig cells following chemical inhalation have been found in the literature. In this study, the staining intensity of RLF produced from Leydig cells, histomorphometric
changes (seminiferous tubule area, seminiferous epithelial height) of testicular tissues obtained from rats and variations in body weight and testes weight of rats which were exposed to xylene and formaldehyde from the beginning of the embryonic period, after birth (1-day-old) and immediately after puberty were examined.

MATERIAL AND METHODS

Animals and experimental design

All studies with animals described in this article were reviewed and approved by the University of Adnan Menderes Institutional Animal Ethics Committee.

For the experiments, 96 male Sprague-Dawley rats that were bred for 7 years as a closed colony at the Experimental Animal Resources and Research Unit of Veterinary Physiology were used. At the beginning of the experiment, the rats were randomly divided into three groups with the following ages: embryonic day 1 (beginning of the embryonic period) (Group I), 1-day-old infantile rats (Group II) and adult rats (Group III), each containing 32 animals. Group I was established as follows: nulliparous female rats were housed overnight with adult males (one male to three females) from the same strain and supplier. The day that vaginal smears were found to be sperm-positive was considered day 1 of the embryonic day. Embryos in this group were subjected to formaldehyde and xylene in the maternal uterus by exposing the pregnant rats. After birth of the animals, they were continuously exposed to the toxic gases for 7 days (8 h/d) per week for 5 weeks. Newborn rats were kept together in the same cage with their mothers. The 1-day-old infantile rats (Group II) were also kept with their mothers.

Exposure design

Inhalation exposure procedures have been described by Valentine and Kennedy (Valentine and Kennedy, 2001). The whole body of the rats was exposed to the solvents. To acclimatize, the rats were housed in a closed chamber fitted with 4 rat cages, made of glass and stainless-steel, with a set of 8 animals per cage under standard laboratory conditions (light period 6:00 to 20:00 h, 24±1°C, tap water and standard pellet diets (Best Yem, Gebze, Turkey) were given ad libitum) for 8 weeks. These chambers were operated dynamically with filtered air at a flow rate providing the necessary amount of gases to the animals from formaldehyde (6 ppm) (Monticello et al., 1989), technical xylene (300 ppm) (Li et al., 1986), and a combination of formaldehyde and technical xylene (3 ppm+150 ppm) for 8 h per day (between 9:00 and 17:00 h) during an 8 week period (Sandikci et al., 2007). Levels of formaldehyde and xylene in cages were monitored by gas detection pumps by the methods of Norback et al. (1995), (Leben Dragger, Accuro® Gas Detection Pump, Arta-F001-6400.00; for formaldehyde: 2/a Batch-ARTA-0351-8101751, for xylene; 10/a Batch-ARSD-0632-6733161) (formaldehyde (Sigma CAS No: 50-00-0) and technical xylene (Sigma CAS No: 1330-20-7); Sigma Chemical Co., St Louis, Missouri, USA). The position of the cages within the chamber was systematically changed each week. Fresh air was provided at a constant temperature. Control animals were placed in an identical chamber without the addition of these two agents.

Sample collection, preparation, triple staining and immunohistochemistry

The rats were killed 5, 8 and 18 weeks after the exposure period for Groups I, II and III, respectively. Animals were weighed and body weights were determined. Rats were anesthetized with xylazine (2% Alfazyme, Ege Vet) and ketamine (10% Alfarmyne, Ege Vet) and subsequently euthanized. Then, testes of the rats were removed and their total weights were recorded. Subsequently, the testes were fixed in 10% neutral buffered formaldehyde/NBF for 24 hours and both testes were blocked together in paraffin following routine tissue follow-up procedures.

Four serial transverse sections (6 µm thickness) were collected at 50 µm intervals from the prepared paraffin blocks for each animal on a rotary microtome, and mounted on slides. The triple staining method was applied to three serial sections to determine histomorphometric changes (seminiferous tubule area, seminiferous epithelial height) (Culling et al., 1985). In the present study, the expression of RLF in the rat testis was investigated by an immunohistochemistry-sABC staining method using a specific antibody-Rabbit anti- Ins13 antiserum (Phoenix Pharmaceuticals, Inc., H- 035-43) raised against rat RLF in one section for each animal (Hsu et al., 1981).

Measuring of the seminiferous tubules

The slides were examined with a light microscope (Leica DMLB) equipped with an image analysis system (Leica Q win standard). For each animal, the area and epithelial height of 10 round or nearly-round pieces of the seminiferous tubule were measured in each of the three sections which had been triple dyed. The epithelial height of the seminiferous tubule was measured from the boundary layer to the junction of the seminiferous tubule epithelial and tubular lumen.

Measurements were made interactively with the help of an image analysis program (Leica Q win standard) linked to a computer.
Measuring of intensity of RLF

The sections to which the sABC staining method was applied were examined and a subjective assessment of intensity of RLF in the tissue was made. The appropriate parts of the examined sections were photographed.

Statistical analyses

The statistical package SPSS (for Windows 11.5) was used for statistical analysis of the data. Differences between each age group with respect to body weight, testes weight, tubule area and epithelial height were analyzed utilizing one-way analysis of variance (ANOVA). Determination of the group source of the difference was done with the Duncan’s test. Differences were considered significant when P values were less than 0.05.

Searching for differences between all age groups with respect to the examined parameters was assessed using the general linear model (GLM) by accepting body weight as the covariance-factor.

RESULTS

Histological findings were examined in the control group. The lumen of the seminiferous tubule in the testes of Group I was observed to be clear and spermatogenic cells were seen to form a thick layer (Figure 1A). Sertoli cell cytoplasm was observed in the tubules of all age groups in the present study and could be sporadically differentiated. Spermatids were observed in Group I and II control groups. A decrease in epithelial height of the seminiferous tubule and Sertoli cell cytoplasm differentiation was clearly seen in Group III (Figure 1B). Spermatozoa were clearly observed in the lumen of the tubule. Leydig cells were detected in all groups.

RLF(+) cells (Figure 2A, B) were determined by immunohistochemically-sABC staining. RLF was determined in all control groups. Little staining intensity was noticed in Groups II and III exposed to formaldehyde, whereas RLF was intensively stained in all age groups and no influence of xylene inhalation was observed. RLF was stained very intensely in Group I exposed to xylene-formaldehyde inhalation; however, no staining was observed in Groups II and III (Table 1).

Group I

Body weight, testes weight, area of tubules and
epithelial height values determined in Group I rats are shown in Table 2.

Decreases observed in body weight and testes weight of rats in experiment groups were determined to be insignificant when compared with those of the control group. Chemical exposure in Group I was observed to cause a significant decrease (p<0.001) in the area of tubules (Figure 3A), and the difference observed between xylene and formaldehyde and xylene and xylene-formaldehyde was significant (p<0.001) (Table 2). Area of tubules was found to be the smallest in Group 1 exposed to xylene-formaldehyde.

The effect of body weight on epithelial height was insignificant. Exposure to all three chemicals caused a significant decrease (p<0.001) (Table 2) of seminiferous tubule. Furthermore, formaldehyde was more effective than xylene, and xylene-formaldehyde was much more effective than either xylene or formaldehyde (p<0.001) (Table 2). Area of tubules was found to be the smallest when exposed to xylene-formaldehyde. Epithelial height was at the highest level in Group I control. Epithelial height of all Group I, including all chemical substance groups, was higher than that of other age groups.

### Group II

When body weight of Group II control rats was compared to those of rat groups treated with chemicals, only the body weight of rats exposed to xylene-formaldehyde was significantly heavier than other groups (p<0.01) (Table 3).

Chemical inhalation did not significantly affect testes weight and area of tubules. A significant decrease in epithelial height was observed only in the formaldehyde group (p<0.001) (Table 3) (Figure 3B).

### Group III

No statistical difference was detected when body weight and testes weight of Group III rats were compared to those of rat groups exposed to chemicals (Table 4).

Area of tubules was found to be the largest in Group III exposed to formaldehyde. In terms of area of tubules, mean values of groups to which formaldehyde and xylene-

### Table 1.

Staining intensity of RLF in the testes belonging to five (Group I), eight (Group II) and eighteen (Group III)-week-old control and exposed to xylene, formaldehyde and xylene-formaldehyde groups

|                  | Group I | Group II | Group III |
|------------------|---------|----------|-----------|
| Control          | ++++    | +++      | ++++      |
| Xylene           | +++     | +++      | +++       |
| Formaldehyde     | +++     | +        | ++        |
| Xylene-Formaldehyde | +++    | -        | -         |

- : No staining, +: Very weak staining, ++: Weak staining, +++: Moderate staining, ++++: Strong staining.

### Table 2.

Body weight, testes weight, area of tubules and epithelial height values belonging to five-week-old control and exposed to xylene, formaldehyde and xylene-formaldehyde groups (Table 2)

|                          | Control | Xylene | Formaldehyde | Xylene-Formaldehyde | p     |
|--------------------------|---------|--------|--------------|----------------------|-------|
| Body weight (g)          | 49±3    | 44±3   | 48±3         | 47±4                 | NS    |
| Testes weight (g)        | 0.6±0.1 | 0.5±0.1| 0.6±0.1      | 0.5±0.1              | NS    |
| Tubules area (μm²)       | 22,940±334<sup>a</sup> | 19,028±412<sup>b</sup> | 17,997±293<sup>c</sup> | 17,884±241<sup>c</sup> | ***   |
| Epithelial height (μm)   | 57±0.6<sup>a</sup> | 52±0.5<sup>b</sup> | 49±0.5<sup>c</sup> | 44±0.4<sup>d</sup>   | ***   |

<sup>a,b,c</sup> Means within each grouping with different letter designations differ significantly.

NS = No significant, *** p<0.001.

### Figure 3.

Appearance of the tubules of the testis tissue belonging to five (A) and eight-(B)-week-old exposed to formaldehyde and eighteen-week-old (C) exposed to xylene-formaldehyde groups. Triple staining method. (A) Formaldehyde caused a significant decrease (p<0.001) in the area of tubules in five-week-old group (Group I). (B) Epithelial height decreased in eight-week-old group (Group II) exposed to formaldehyde (p<0.001). (C) Epithelial height increased significantly in eighteen-week-old group (Group III) exposed to xylene-formaldehyde (p<0.001).
formaldehyde were applied were significantly higher than those of the control and xylene-applied rats (p<0.001). However, the height of tubular epithelium was observed to increase significantly only in the group to which xylene-formaldehyde was applied (p<0.001) (Table 4) (Figure 3C).

In statistical examination performed between groups to which chemical was applied; a significant increase in area of tubules was observed in formaldehyde and xylene-formaldehyde groups in comparison to the xylene group (p<0.001) (Table 4). However, in terms of epithelial height significant increases were determined in formaldehyde, xylene, and xylene-formaldehyde groups (p<0.001).

The effect of age on values of body weight, testes weight, area of tubules and epithelial height in control groups

Values of body weight, testes weight, area of tubules and epithelial height determined in control rats in different age groups are shown in Table 5.

It was observed that body weight increased with age. Weight difference between Group III and Group I and Group II was statistically significant (p<0.001).

Testes weight also increased with age (p<0.001). In terms of area of tubules the highest values were determined in Group III rats. The effect of age on area of tubules was significant (p<0.001). The mean value for area of tubules in Group II was lower than in Group I and Group III rats.

Effect of age on epithelial height was statistically significant also (p<0.001). Mean epithelial height of Group I was found to be significantly higher than in Group II and Group III rats (p<0.001).

The effect of age, body weight and chemical inhalation on testes weight, area of tubules and epithelial height

The effect of age, body weight and chemical inhalation on testes weight, area of tubules and epithelial height and interactions between chemical substance and age are shown in Table 6 and 7.

The effect of age and body weight on testes weight was statistically significant (p<0.001); however, the effects of chemical inhalation and interaction between chemical substance and age were insignificant. Testes weight, independent from chemical inhalation in all groups, increased with advancing age and body weight (Table 6).

When the effect of age was eliminated, the effect of Table 5. Body weight, testes weight, area of tubules and epithelial height values determined in rats of different age in control groups (X ± S_x)

| Age    | Control | Xylene | Formaldehyde | Xylene-Formaldehyde | p       |
|--------|---------|--------|--------------|----------------------|---------|
| 5 weeks| 49±3    | 77±4   | 193±5        | 186±5                | NS      |
| 8 weeks| 0.6±0.1 | 1.6±0.1| 3.8±0.2      |                      | NS      |
| 18 weeks| 22,940±334 | 18,339±295 | 25,523±409 | 25,523±409 | ***     |

**a, b, c** Means within each grouping with different letter designations differ significantly. NS = No significant, *** p<0.001.
body weight and chemical substance on the area of tubules was statistically insignificant (Tables 6 and 7). Tubule area was found to be largest in Group III exposed to formaldehyde and smallest in Group I exposed to xylene-formaldehyde. In chemical-exposed groups, tubule areas were larger in Group III than in other age groups (Table 6).

The effect of body weight on epithelial height was insignificant; however, the effect of age, chemical substance and age, and chemical substance interactions were significant (p<0.001) (Tables 6 and 7). Epithelial height was at the highest level in Group I and II controls, but was high only in Group III exposed to xylene-formaldehyde. In all chemical substance groups, epithelial height was the highest only in Group I (Table 6).

When the effect of chemical substance on epithelial height was examined (p<0.001), no statistical significance was determined between control and xylene; formaldehyde and xylene-formaldehyde groups. Epithelial height in control and xylene groups was statistically more significant than formaldehyde and xylene-formaldehyde groups. Although the increase in the epithelial height of Group III was statistically insignificant, it was more significant in Group I than in Group II and III (p<0.001) (Table 7).

**DISCUSSION**

**Histological appearance**
Orth (1982) reported that the proliferation period of Sertoli cells in the rat continued until postnatal days 15-20. In the present study, Sertoli cell cytoplasm was observed in the tubules of all age groups. Yang et al. (1990) reported that round spermatids were first seen at the 25th day, rapidly

**Table 6.** The effect of body weight, age, chemical inhalation, and chemical substance-age interaction on testes weight, area of tubules and epithelial height values determined in rats of different age groups exposed to xylene, formaldehyde and xylene-formaldehyde (X ± S_\text{p})

| Age      | Control | Xylene | Formaldehyde | Xylene-Formaldehyde |
|----------|---------|--------|--------------|---------------------|
| Testes weight (g) | 5 weeks | 0.6±0.2 | 0.5±0.2 | 0.6±0.2 | 0.6±0.3 |
|          | 8 weeks | 1.6±0.4 | 1.7±0.1 | 1.7±0.1 | 1.8±0.6 |
|          | 18 weeks | 3.8±0.4 | 3.9±0.3 | 3.6±0.1 | 3.7±0.3 |
| Tubules area (μm²) | 5 weeks | 22,940±3,002 | 19,028±5,759 | 17,997±3,751 | 17,84±2,070 |
|          | 8 weeks | 18,339±3,413 | 17,995±2,635 | 17,981±2,033 | 17,991±3,823 |
|          | 18 weeks | 25,523±3,372 | 25,035±6,549 | 28,960±14,909 | 28,083±3,432 |
| Epithelial height (μm) | 5 weeks | 57±3 | 52±4 | 49±4 | 44±2 |
|          | 8 weeks | 40±2 | 39±4 | 36±1 | 39±2 |
|          | 18 weeks | 41±2 | 42±5 | 40±3 | 43±6 |

**Table 7.** The effect of body weight, chemical substance, age and chemical substance-age interaction on mean testes weight, area of tubules and epithelial height values determined for all age groups exposed to xylene, formaldehyde and xylene-formaldehyde (X ± S_\text{p})

| Age      | Testes weight (g) | Tubules area (μm²) | Epithelial height (μm) |
|----------|-------------------|--------------------|------------------------|
| 5 weeks  | 1.18±0.07^a       | 21,392±2,086       | 52±1.2^a               |
| 8 weeks  | 1.90±0.04^b       | 18,507±1,193       | 38±0.1^b               |
| 18 weeks | 2.95±0.09^a       | 24,369±2,599       | 41±1.0^b               |
| Body weight | ***             | NS                 | ***                    |
| Chemicals | NS                | NS                 | ***                    |
| Control  | 2.00±0.04         | 22,358±1,168       | 46±0.7^a               |
| Xylene   | 2.04±0.04         | 20,656±1,165       | 45±0.7^a               |
| Formaldehyde | 2.01±0.04     | 21,751±1,169       | 42±0.7^b               |
| Xylene-Formaldehyde | 1.98±0.04 | 20,925±1,238       | 42±0.7^b               |

Means within each grouping with different letter designations differ significantly.

NS = No significant, *** p<0.001.
increased between the 25th-40th days and continued to increase until the 70th day in Sprague-Dawley rats. Spermatids, becoming more clear starting from eight weeks of age, were noticed in all age groups also in this study.

Leydig cells were detected in all age groups. In another study (Ge et al., 2005), postnatal development of Leydig cells was divided into three periods. Firstly, progenitor cells were found in 14 and 21 day testes. Later, cells converted into immature Leydig cells (Immature Leydig Cells, ILC) until day 35 by gaining steroidogenic organelle structure and enzyme activity, but most of the testosterone produced by these cells was metabolized. Lastly, following this period adult Leydig cells were formed until 90 days of age (Adult Leydig Cells, ALC). The newly formed cells actively produced testosterone. When the findings of Ge et al. (2005) were used as reference points, immature Leydig cells were seen in Group I and II rats and adult Leydig cells were seen in Group III, which were the material of the present study.

Relaxin-like factor

RLF was observed in the progenitor Leydig cells of rat testes for the first time after day 25 (Ge et al., 2005; Sadeghian et al., 2005). Leydig cells were determined RLF positive in all control groups. When evaluated subjectively, decreased staining intensity was observed in Group II and III, but no staining was seen in Group II and III exposed to xylene-formaldehyde.

In a study performed by Fay et al. (1995) no variation in serum testosterone level was determined following xylene inhalation; however, Yamakada (1993) determined a decrease in serum testosterone levels. In the present study, RLF was intensively stained and none of the three age groups exposed to xylene was observed to be affected by xylene.

RLF was intensely detected in Group I exposed to formaldehyde; however, staining intensity decreased in Group II and III. Consequently, rats were negatively affected by formaldehyde inhalation eventuating in the postnatal period. Ozen et al. (2003) reported that serum testosterone levels decreased in Wistar rats exposed to formaldehyde. Decline in RLF intensity in Group II and III exposed to formaldehyde may be connected to the decline in Leydig cell functions.

RLF staining intensity in Group I exposed to xylene-formaldehyde was observed to be very intense; however, no staining occurred in other age groups. We can conclude that there is no influence of xylene inhalation upon RLF staining; on the contrary, there is an influence of formaldehyde, consequently formaldehyde is effectual in the xylene-formaldehyde combination.

Age, body weight and the effect of chemical inhalation

on testes weight, area of tubules and epithelial height

Testes weight: In studies conducted on rats, Cassidy et al. (1983) and Shah et al. (1987) observed no significant variation in testes weight and body weight with formaldehyde application (Cassidy et al., 1983; Shah et al., 1987). On the other hand, several researchers determined that formaldehyde led to a decrease in body weight (Khan et al., 2003) and testis weight (Khan et al., 2003; Zhou et al., 2006).

There have been studies reporting no variations in body weight (Nylen et al., 1989) and testis weight (Nylen et al., 1989; Fay et al., 1995) of rats subsequent to xylene inhalation. In contrast, as a result of xylene inhalation in rats, Yamakada (1993) determined a decrease in body weight and testis weight. In the present study, the effects of body weight and age upon testes weight were found statistically significant (p<0.001) (Table 6 and 7); however, chemical substance inhalation was observed to be ineffective on testes weight.

Area of tubules: Khan et al. (2003) and Ozen et al. (2003) reported significant decreases in the seminiferous tubular diameter in studies performed on the effects of formaldehyde on the cock and rat testis tissue. Different outcomes were obtained in the present study. Chemicals exposure in Group I and III caused a significant decrease (p<0.001) in the area of tubules. However, chemical inhalation did not significantly affect testes weight and area of tubules in Group II. When the effects of age, body weight, chemical substance and interaction between chemical substance and age on mean area of tubules were examined, they were found to be statistically insignificant (Table 7).

Epithelial height: Although the effect of body weight on epithelial height was insignificant, the effects of age, chemical substance and interaction between chemical substance and age was significant (p<0.001). When the effect of chemical substance upon epithelial height was examined, epithelial height of control and xylene groups was found to be significantly higher than that of the formaldehyde and xylene-formaldehyde groups (p<0.001) (Table 6). Xylene did not affect epithelial height; however, formaldehyde and xylene-formaldehyde inhalations reduced epithelial height. Zhou et al. (2006) also reported that intraperitoneal administration of formaldehyde in adult rats (208-216 g) caused reduction in seminiferous epithelial height. However, Tang et al. (2003) determined that formaldehyde inhalation caused a reduction in seminiferous epithelial height in 6-14 day-old mice also.

Epithelial height was higher in Group I control and treatment groups than in Group II and III (p<0.001). Although mitosis and apoptosis were active due to the first spermatogenetic wave in Group I rats, in Group II animals there was balance since the first spermatogenetic wave was
completed. Normal spermatogenesis was continuing in Group III rats. So the highest epithelial height detected in Group I may originate from the first spermatogenetic wave.

It has been shown that xylene and formaldehyde toxicity can increase the production of reactive oxygen species (ROS) in testicular tissue. ROS including singlet oxygen, hydrogen peroxide, superoxide anions and hydroxyl radicals are important mediators of cellular injury and play an important role in oxidative damage. Oxidative stress is an important mechanism of testicular damage. Excessive ROS increases apoptosis of germ cells and inhibits the activity of spermatozoa (Ozen et al., 2005; Zhou et al., 2006; Kus et al., 2008).

In conclusion, formaldehyde and xylene-formaldehyde combination negatively affected rat testes tissue in terms of Leydig cells (RLF) and tubule epithelia. These findings are important with regard to their use as reference data for the histological, histopathological and endocrinological studies which will be performed on this topic and their contribution to scientific knowledge.

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