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QUANTITATIVE STUDIES ON COMPENSATORY TESTICULAR HYPERTROPHY IN PIGS AFTER UNILATERAL CASTRATION AT VARIOUS AGES

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Summary

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A morphometric study of testes from pigs at various ages was performed. Six animals for each age group (day 1, months 1, 2, 3, 4, 5 and 6) were unilaterally castrated and the removed testes were used as controls. The definitive orchiectomy was performed two months after the first castration and the obtained testes formed the experimental groups. Testes, obtained following bilateral castration of three pigs aged 7-8 months were further added to controls.

The unilateral castration resulted in significant testicular hypertrophy of the remaining testis up to the age of 4 months while in 5- and 6-months old animals the increase in volume and weight of experimental organs was statistically insignificant compared to controls. The compensatory testicular hypertrophy, evaluated via the weight corresponded to a bigger diameter and area of seminiferous tubules of experimental testes.

Key words: compensatory hypertrophy, morphometry, pigs, testes, unilateral castration

INTRODUCTION

The unilateral castration (UC) results in a compensatory enlargement of the remaining testis because of the impaired dynamic interactions in the hypothalamo-pituitary-testicular system. A compensatory testicular hypertrophy was described in various animal species: rats (Brown et al., 1991), rabbits (Mirando et al., 1984), rams (Voglmayr and Mattner, 1968), goats (El-Barody et al., 1997), bulls (Johnson, 1978), stallions (Ott et al., 1984) with extent, dependent on the age of the unilateral castration.

The studies in pigs are incomplete and in most cases refer to sexually immature animals. Most frequently, the weight of the remaining testis was determined. It was observed that after UC at the age of 7 days, the weight of the remaining testis reached 86% at 31 days (Clark et al., 1996) and 95% at 40 days (Thomford and Dzuik, 1984) of the corresponding weight of both intact testes. After UC at the age of 28 days, the weight of the remaining testis attains up to 80% of the weight of both testes at the time of slaughtering of animals, with live weight about 125 kg (Greger and Hagen, 1996).

The present study was motivated by the insuffucient data for the reaction of the

remaining testis to UC of pigs at various ages. Furthermore, we aimed to determine some histometric parameters that are important for the development of testes.

MATERIALS AND METHODS

The experiments were performed on 48 male pigs, Large White & Landrace crossbreds, originating from the specialized pig-breeding farm, Yambol. Six pigs were unilaterally castrated at each of the following ages - 1 day, 1, 2, 3, 4, 5, and 6 months. The second (definitive) orchiectomy was performed 2 months following the first one. The testes obtained from the UC were used as controls and those from the definitive orchiectomy - as experimental. The control group was completed with 3 pigs at the age of 7 and 8 months respectively, castrated bilaterally. Immediately after the operation, the testes were weighed and their volume was determined. The material for the hstological study was taken from the equatorial part of each testis. The specimens were fixed in *ex tempore* prepared Bouin's liquid, embedded in paraffine and cut with a paraffine microtome. The obtained cross sections, 6 μm thick were stained with haematoxillin-eosin. The ready histological preparations were examined with a HUND microscope.

The following morphometric parameters were determined:

* weight of testes (g) - by an analytical balance;
* volume of testes (mm^3) – by measuring the amount of displaced water in a glass cylinder;
* diameter of seminiferous tubules (μm) - by a standardized eye-piece micrometer;
* area of seminiferous tubules (S, μm^2) - according to the equation:

$$S = 0.25\pi \times d^2$$

where: d = mean diameter of seminiferous tubules in μm;

* relative volume of seminiferous tubules and the interstitium (%) - according to Pakenas (1968);
* percentage of compensatory hypertrophy (% CH) - according to the equation:

$$\% CH = \frac{(We-Wc)}{Wc} \times 100$$

where: We= weight of experimental testis; Wc = weight of the control testis.

The results were statistically processed using the t-criterion of Student.

RESULTS

Development of testes by weight and volume

The weight of control testes increased 260 times (from 0.98 to 261.67 g - Table 1) up to the age of 8 months. At each studied age up to 4 months, the weight of testes was almost double compared to that of the previous measurement. At the age of 5 months the respective increase was almost triple. Then followed a less intensive increase in testicular weight up to the age of 8 months (on the average by 60% at 6 and 7 months and only by 13% between 7-8 months).

The average weight of the experimental group of testes was double up to the age of 3 and 4 months, triple at the age of 5 months, bigger by 30% at 6 and 7 months and by 8% at 8 months compared to that at the previous observed age group.

The differences between groups at the various ages were highly significant ($p<0.001$) up to 6 months and less significant at 7 and 8 months ($p<0.01$ and $p<0.05$ respectively).

Table 1. Weight of testes (g) prior to and 2 months after unilateral castration at various age and percentage of compensatory hypertrophy. Data are presented as mean ± SEM

Age	Control group of testes[1] (n=6)	Experimental group of testes[2] (n=6)	Percentage of compensatory hypertrophy
1 day	0.98 ± 0.06	-	-
1 month	3.17 ± 0.09	-	-
2 months	8.07 ± 0.14	15.25 ± 0.21 ***	89.05
3 months	14.13 ± 0.19	28.58 ± 0.35 ***	102.22
4 months	30.28 ± 0.74	57.08 ± 1.12 ***	88.52
5 months	88.42 ± 0.98	149.17 ± 4.73***	69.23
6 months	143.83 ± 2.82	184.83 ± 2.29***	28.50
7 months	231.17 ± 3.29	255.83 ± 3.04 **	10.67
8 months	261 ± 2.95	272.50 ± 3.09*	4.14

[1] testes removed at the first (unilateral) castration; [2] testes removed 2 months after the unilateral castration. * $p<0.05$; ** $p<0.01$; *** $p<0.001$ vs control group of testes.

Table 2. Volume of testes (mm^3) prior to and 2 months after unilateral castration. Data are presented as mean ± SEM

Age	Control group of testes[1] (n=6)	Experimental group of testes[2] (n=6)
1 day	1.12±0.06	-
1 month	3.23±0.09	-
2 months	8.25±0.11	15.60±0.17***
3 months	14.35±0.19	29.17±0.42***
4 months	30.72±0.74	57.83±1.04***
5 months	89.25±0.99	153.3±5.14***
6 months	147.00±3.14	188.5±3.11***
7 months	233.83±3.11	258.17±4.30**
8 months	265.83±3.05	277.17±3.04*

[1] testes removed at the first (unilateral) castration; [2] testes removed 2 months after the unilateral castration.* $p<0.05$; ** $p<0.01$; *** $p<0.001$ vs control group of testes.

The compensatory hypertrophy of experimental testes up to the age of 4 months was clearly manifested while that following the unilateral castration after the age of 5 months was much lesser expressed (Table 1).

The results of testicular volume determinations (Table 2) were similar to the tendency observed in weight change. The data of both groups showed a various level of statistical difference: $p<0.001$ up to 6 months; $p<0.01$ at 7 months and $p<0.05$ at 8 months.

Histomorphometric study

The diameter of seminiferous tubules (Table 3) increased insignificantly from the 1st day to the 3rd month in control group. The biggest increase in the diameter vs that at the previous observed age was noticed at the age of 4-5 months (about 60%) that corresponded to the onset of sexual maturity. Then followed a gradual increase by about 20% at the age of 6, 7 and 8 months.

The data of the experimental group showed that the most significant enlargement of seminiferous tubules occurred between months 3 and 4 (by about 60%) that was related to the earlier beginning of spermatogenesis and the earlier sexual maturity. Afterwards the diameter increased by about 10% between age intervals up to the end of the study. The differ-

ences in that parameter between groups were statistically significant up to the age of 5 months ($p<0.001$) and at 6 and 7 months ($p<0.01$).

Table 3. Diameter of seminiferous tubules (μm) prior to and 2 months after unilateral castration. Data are presented as mean ± SEM

Age	Control group of testes [1] (n=6)	Experimental group of testes[2] (n=6)
1 day	57.70±0.45	-
1 month	60.63±1.28	-
2 months	67.57±1.83	86.07±1.54***
3 months	76.93±1.55	104.80±2.28***
4 months	94.80±1.47	174.00±1.40***
5 months	146.67±4.83	193.40±1.50***
6 months	187.33±1.81	204.50±2.55**
7 months	215.24±1.60	226.67±1.26**
8 months	239.03±0.94	242.57±2.22

[1] testes removed at the first (unilateral) castration; [2] testes removed 2 months after the unilateral castration. ** $p<0.01$; *** $p<0.001$ vs control group of testes.

The area of seminiferous tubules (Table 4) was bigger in the experimental group. The differences vs controls were signigficant at the level of $p<0.001$ up to the age of 5 months, at $p<0.01$ at 6 and 7 months and insignificant at 8 months.

The data for the relative volume of seminiferous tubules are listed in Table 5. The corresponding differences to 100% are the interstitial percentages. There again, the higher values were those of the experimental testicular group with statistically significant differences vs controls at the ages of 3 and 4 months ($p<0.01$).

DISCUSSION

The morphometric data suggest that the UC of young pigs (at the age up to 4 months) resulted in a significant compensatory testicular hypertrophy. Two months after the hemicastration, this hypertrophy was characterized by almost double weight and volume of the remaining testis and significantly enlarged diameter and area of seminiferous tubules.

Table 4. Area of seminiferous tubules (μm^2) prior to and 2 months after unilateral castration at various age. Data are presented as mean ± SEM

Age	Control group of testes [1] (n=6)	Experimental group of testes [2] (n=6)
1 day	2614.7 ± 46.51	-
1 month	2892.3 ± 124.61	-
2 months	3593.5 ± 194.26	5824.0 ± 203.91***
3 months	4655.5 ± 188.33	8642.2 ± 386.57***
4 months	7063.8 ± 218.56	23774.5 ± 379.65***
5 months	16977.5 ± 1117.5	29370.7 ± 459.39***
6 months	27551.2 ± 440.50	32854.5 ± 813.95**
7 months	36302.2 ± 537.94	40337.8 ± 446.89**
8 months	44855.8 ± 350.89	46207.8 ± 855.14

[1] testes removed at the first (unilateral) castration; [2] testes removed 2 months after the unilateral castration. ** $p<0.01$; *** $p<0.001$ vs control group of testes.

Table 5. Relative volume of seminiferous tubules (%) prior to and 2 months after unilateral castration at various age. Data are presented as mean ± SEM

Age	Control group of testes[1] (n=6)	Experimental group of testes[2] (n=6)
1 day	21.6 ± 1.94	-
1 month	22.8 ± 1.49	-
2 months	32.0 ± 2.28	33.2 ± 1.36
3 months	41.2 ± 2.87	55.2 ± 1.65**
4 months	54.8 ± 3.81	70.4 ± 1.47**
5 months	65.6 ± 2.32	72.8 ± 3.50
6 months	72.8 ± 1.62	76.4 ± 1.47
7 months	76.4 ± 2.79	78.4 ± 1.60
8 months	80.4 ± 1.60	81.6 ± 2.04

[1] testes removed at the first (unilateral) castration; [2] testes removed 2 months after the unilateral castration. ** $p<0.01$ vs control group of testes.

The unilateral castration at the age of 5 and 6 months was accompanied by a lesser increase in both the weight and volume of the remaining testis. This fact as well as the weaker enlargement of seminiferous tubules give the reason to assume that the ability for compensatory hypertrophy was decreased.

In pigs aged between 3 and 4 months, the proliferation of Sertoli cells and the morphological differentiation of their nuclei ends (Van Straaten and Wensing, 1977). At this age, the haematotesticular barrier in this animal species is formed as well. Considering our results, we suggest that those are among the factors determining the sensitivity of testes to the removal of the mated organ.

Our data correspond to those of Boockfor et al. (1983) that reported a much more stronger compensatory testicular hypertrophy following UC of calves at the age of 3 months compared to UC at the age of 6 and 9 months. The testicular reaction to UC in rats is also age-dependent: the critical age, after which the removal of one testis doesn't result in compensatory hypertrophy of the other is 20 days (Cunningham et al., 1978; Klaij et al., 1994).

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