ENDOCRINE PROFILES, TESTICULAR GONADOTROPIN RECEPTORS AND SPERM PRODUCTION IN HEMI-CASTRATED RAM LAMBS

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ABSTRACT

The effects of hemi-castration upon compensatory hypertrophy, serum gonadotropin and testosterone concentrations, testicular gonadotropin receptors and daily sperm production (DSP) were studied in 10 crossbred ram lambs. At 4 mo of age lambs were either hemi-castrated (HC; n=5) or left intact (INT; n=5). Blood samples were collected every 2 h for the first 24 h post-surgery, every 6 h for the next 24 h and then three times weekly for the following 14 wk. Serial blood samples (15-min intervals for 8 h) were collected during the 4th, 8th and 12th week following hemi-castration. Individual mean testicular and epididymal weights increased (P<.05) 48 and 33% in HC compared with INT rams, respectively. Serum follicle stimulating hormone (FSH) increased (P<.05) within 8 h after HC, reached peak concentrations within 1 wk and remained elevated for 4 wk before returning to concentrations of INT rams. Neither mean serum luteinizing hormone (LH) nor pulse patterns of LH or FSH were different (P>.05) between these two groups at any period examined. Serum testosterone (T) concentrations were lower (P<.05) during the first 48 h post-surgery in HC rams, but by 1 wk concentrations were similar (P>.05) to those in INT rams. Remaining testes from HC and INT rams were removed at 7 mo of age, 3 mo after initial gonadal manipulation. On a per-testis basis there were more (P<.05) LH and FSH receptors in HC than INT rams, respectively; however, concentrations of receptors were not different (P>.05). Similarly, DSP/testis was greater (P<.05) for HC than INT animals, while DSP/g parenchyma was similar (P>.05). These results indicate: 1) increased serum FSH was related to compensatory hypertrophy, 2) complete compensation of androgen secretion in HC rams was not related to a complete compensation in testicular size, alterations in secretion of serum LH or in changes of testicular gonadotropin receptor concentrations and 3) the increase in DSP in the remaining testis following hemi-castration was not associated with an increase in spermatogenic efficiency of the testicular tissue.

(Key Words: Castration, Gonadotropins, Receptors, Testosterone, Spermatogenesis, Sheep.)

Introduction

Although the phenomenon of compensatory hypertrophy has been recognized and studied for nearly 100 yr, the exact mechanisms responsible for these changes remain elusive. Removal of one testis results in compensatory hypertrophy of the remaining testis in a number of species, including prepubertal and adult rams (Ribbert, 1890; Lipschutz, 1922; Voglmayr and Mattner, 1968; Land and Carr, 1975; Hochereau-de Reviers et al., 1976). Compensatory increases in androgen secretion and sperm production have also been observed (Voglmayer and Mattner, 1968; Johnson et al., 1971; Walton et al., 1978; Boockfor et al., 1983).

It is generally believed that an alteration in the hypothalamic-pituitary-testicular axis occurs following hemi-castration resulting in gonadotropin-stimulated increases in both tubular and intertubular elements of the testis (Hochereau-de Reviers et al., 1976). However, the endocrine data have been somewhat conflicting, even within species. In sheep, neonatal hemi-castration results in increased follicle stimulating hormone (FSH) secretion for 3 to 12 wk following hemicastration without a corresponding change in other pituitary hormones (Walton et al., 1978, 1980; Jenkins and Waites, 1983, Waites et al., 1983). In contrast, others have demonstrated elevations in plasma luteinizing hormone (LH)
for various lengths of time with (de Reviers et al., 1980) or without (Hochereau-de Reviers et al., 1980) significant changes in FSH. Differences among breeds, season and age at hemicastration appear to explain some, but not all, of these discrepancies (Hochereau-de Reviers et al., 1976; Jenkins and Waites, 1983).

In most of the earlier studies infrequent bleeding regimens were used. Because gonadotropins, especially LH, are episodically released (Butler et al., 1972; Lincoln, 1975), subtle changes in pulsatile secretion cannot be detected in daily or weekly samples. One objective of this study was to characterize more critically the short- and long-term effects of hemicastration on LH, FSH and testosterone (T) secretion in prepubertal lambs hemicastrated at 4 mo of age. After these animals attained puberty (7 mo of age), the remaining testes were removed to determine whether compensatory hypertrophy was associated with changes in concentrations of testicular gonadotropin receptors or spermatogenic efficiency (sperm/g tissue).

Materials and Methods

Animals. Ten crossbred (Dorset x Rambouillet) ram lambs, born between January 20 and February 5, 1985 were raised in sheds under conditions of natural photoperiod and temperature. At 4 mo of age the 10 ram lambs were anesthetized (1 mg ketamine-HCl/kg and 1 mg xylazine/kg body weight and 1.5 mg atropine sulfate, im) and either hemicastrated by removing one testis and its associated epididymis (HC; n=5) or left intact (INT; n=5). At 7 mo of age all rams in each treatment group were castrated. Testes and epididymides were weighed immediately after removal. Testicular parenchyma was frozen in liquid nitrogen and stored at -80 C until subsequent analyses.

Blood Sampling. An initial blood sample was obtained from each lamb via jugular venipuncture prior to anesthesia (0 h). Blood sampling was continued at 2-h intervals for the first 24 h, at 6-h intervals for the next 24 h and then three times weekly for 14 wk. On one day during the 4th, 8th and 12th week following hemicastration, indwelling catheters with obturators were placed in a jugular vein and blood samples were collected at 15-min intervals for 8 h. Sera were separated from cells by centrifugation and stored at -20 C.

Radioimmunoassays. Serum LH was analyzed using a previously validated assay (Niswender et al., 1969) with NIH-LH-S18 as standard. Intra- and inter-assay coefficients of variation were 5.6 and 7.8%.

Serum FSH was quantified using a double antibody radioimmunoassay previously used for bovine serum (Acosta et al., 1983). To validate this assay for ovine serum, cross-reactivity of the rabbit anti-ovine FSH antiserum (#178) was determined to be less than 3% for 200 ng LH (NIH-LH-S18), growth hormone (NIH-GH-S11), prolactin (NIH-PRL-S12) or GnRH. Upon addition of 5, 10, 25 and 50 ng ovine FSH to 50 µl of serum with undetectable FSH levels, 4.85, 10.56, 24.00 and 49.75 ng were recovered (y=131 + .987x; r=.99; P<.001). Inhibition curves generated by serial dilutions of castrate sheep serum and the ovine standard (NIH-FSH-S8) were parallel. Assay sensitivity was 1.5 ng/tube. Intra- and inter-assay coefficients of variation were 6.5 and 8.8%.

Serum T was measured in ether-extracted serum using the procedure described by Chakraborty et al. (1984). The first antibody (GDN#250) was pre-absorbed with dihydrotestosterone (DHT), which reduced the cross-reactivity of this antibody with DHT from 70 to 14%. Extraction efficiency was > 90%. Intra- and inter-assay coefficients of variation were 7.5 and 10.8%.

Receptor Assays. Testis LH and FSH receptors were measured in crude testicular membrane preparations as previously described for bovine testis (Melson et al., 1986), using the standard curve technique developed by Nett et al. (1981). The standard curve was generated by incubating 100,000 cpm labeled hormone with 3.8 to 120 mg testicular membranes (wet tissue weight equivalent) per tube. Three dilutions of each testis sample (30, 15 and 7.5 mg/tube) were compared with the standard curve. Parallelism in receptor binding between unknowns and the standard curve indicated that receptors had similar affinities for the labeled ligand. Specific activities of the labeled human chorionic gonadotropin (9,800 IU/mg) and hFSH (NIADDK-hFSH-I-3) were 35 and 10 µCi/µg, respectively. Protein concentrations were determined by the Bradford method (Bradford, 1976) using bovine serum albumin as standard.
Testicular Sperm Numbers. Concentrations of homogenization-resistant spermatids in unfixed testicular tissue were determined using the technique described by Amann and Lambiase (1969). Testicular parenchyma was thawed and homogenized (1 g/10 ml) in a blender at high speed for 5 min in a .15 M NaCl, 3.8 mM NaN₃, .05% (v/v) Triton X-100 solution. Homogenates were stored for 24 h at 4 °C, and spermatid numbers in each testis were determined in four separate hemocytometric counts with an 11.5% coefficient of variation. Estimates of daily sperm production (DSP) were determined by dividing the number of spermatid nuclei in the homogenate by a 3.56-d time divisor, which is the number of days production these reserves represent for the ram (Amann, 1970).

Statistical Analysis. For data presentation and analysis, weekly means consist of the average of their respective three-times weekly values. Hormonal profiles of hourly and weekly means were evaluated using analysis of variance for repeated measures (Steel and Torrie, 1960). Differences in serum FSH were determined by comparing all values to the initial pre-treatment value (0 h) using Dunnett's procedure (Steel and Torrie, 1960). Because of the high degree of sample variability, serum T and LH for the first 48 h (overall mean) and subsequent weekly means were compared using Student's t-tests. Differences in testicular receptor and sperm production means were determined by Student's t-tests. Left and right testicular characteristics in control rams were not different (P>.05) and therefore averaged for all further statistical comparisons.

Basal serum LH and FSH concentrations of samples collected during the 8-h windows were determined by an iterative process similar to that described by Melson et al. (1986), in which high values were excluded until the mean plus three standard deviations (SD) was greater than the remaining values. Hormone pulses were defined as 1) having an amplitude greater than three SD of mean basal levels, 2) occurring within two samples of a previous nadir and 3) containing at least two points on the descending portion of the peak. Pulse amplitude was defined as the highest point associated with the peak minus the mean basal concentration.

Results

Hemi-castration at 4 mo of age did not alter (P>.05) body weight at 7 mo of age, but increased (P<.05) individual testicular and epididymal weights 48 and 33%, respectively (table 1).

Concentrations of serum FSH were similar (P>.05) between INT and HC animals (30.4 ± 3.9 and 34.6 ± 5.2 ng/ml, respectively) at 0 h (prior to anesthesia; figure 1). Mean serum FSH increased (P<.05) within 8 h post-surgery in HC rams, reached peaks of 79.8 ± 12.7 ng/ml at 1 wk, and declined to pre-surgery concentrations by 5 wk. Serum FSH concentrations in INT rams were unchanged (P>.05) throughout the 14-wk period. Although highly variable, serum concentrations of LH were not different (P>.05) between HC and INT rams during the hours or weeks post-surgery (figure 2).

Mean serum T concentrations in HC rams decreased precipitously within 4 h post-hemi-castration and overall means during the first 48 h post-surgery were lower (P<.05) in HC rams (.5 ± .1 ng/ml) compared with INT rams (1.2 ± .1 ng/ml; figure 3). By 1 wk post-surgery, T concentrations were not different (P>.05) between these two groups. Testosterone increased with advancing age in both groups during the study and was higher (P<.05) during wk 14 (3.6 ± .5; 3.5 ± .5 ng/ml) than wk 1 (1.4 ± .3; 1.5 ± .3 ng/ml) for HC and INT rams, respectively.

Pulsatile LH release was observed when blood samples were collected at 15-min intervals.
TABLE 1. MEAN (± SE) BODY, TESTIS AND EPIDIDYMAL WEIGHTS AND DAILY SPERM PRODUCTION (DSP) IN INTACT (INT) AND HEMI-CASTRATED (HC) LAMBS

<table>
<thead>
<tr>
<th>Item</th>
<th>INT</th>
<th>HC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body wt, kg</td>
<td>53.2 ± 1.1</td>
<td>52.2 ± 4.1</td>
</tr>
<tr>
<td>Testis wt, g</td>
<td>127.7 ± 13.6</td>
<td>189.3 ± 9.4*</td>
</tr>
<tr>
<td>Epididymal wt, g</td>
<td>19.9 ± 1.1</td>
<td>26.5 ± 1.4*</td>
</tr>
<tr>
<td>DSP/testis X 10⁶</td>
<td>35.7 ± 2.9</td>
<td>35.8 ± 2.7</td>
</tr>
<tr>
<td>DSP/testis X 10⁹</td>
<td>4.3 ± 0.6</td>
<td>6.3 ± 0.6*</td>
</tr>
</tbody>
</table>

*P<.05.

during the 4th, 8th and 12th week of the study (figure 4). Pulse frequency and amplitude were similar (P>.05) between HC and INT rams and were not different (P>.05) among the three bleeding periods. Overall LH pulse frequency was 2.3 ± .2 and 2.2 ± .2 pulses/8 h and pulse amplitude was 8.8 ± 2.0 and 5.5 ± 1.0 ng/ml for HC and INT rams, respectively. Serum FSH was not secreted in a distinct episodic pattern, and pulses could not be identified using the criteria defined above (figure 4).

On a per-testis basis, DSP was increased 48% (P<.05) in HC rams, but DSP was similar (P>.05) between HC and INT rams when expressed on a per-gram-testis basis (table 1). Similarly, testicular LH and FSH receptor content was increased 55 and 56%, respectively, by hemi-castration (P<.05); however, LH and FSH receptor concentration was unaltered (P>.05; table 2).

Discussion

The testicular hypertrophy observed in this study was not as great as that reported in lambs hemi-castrated at younger ages (Walton et al., 1978, 1980; Hochereau-de Reviers et al., 1980; Waites et al., 1983). Compensatory testicular hypertrophy in those studies typically reached 80 to 100% or greater by 8 to 12 wk post-surgery. The degree of hypertrophy observed in this study more closely resembled that seen in adult rams (Johnson et al., 1971; Hochereau-de Reviers et al., 1976; de Reviers et al., 1980; Mirando et al., 1986), suggesting that there are age-related differences in the gonadal sensitivity to the factors controlling compensatory hypertrophy. An increase in Sertoli cell number has been suggested to contribute to this hypertrophy in neonatal lambs (de Reviers et al., 1980; Waites et al., 1983), while increases in Sertoli cell size and spermatogenic yield are
Figure 4. Representative profiles of serum LH and FSH secretion in a hemi-castrated (5382) and intact (5404) ram. Samples were collected at 15-min intervals for 8 h during the 4th, 8th and 12th week of the study.

more likely responsible for this compensation in older animals (Hochereau-de Reviers et al., 1976; Mirando et al., 1986).

Circulating T concentrations decreased immediately following hemi-castration, but quickly recovered to concentrations not different from INT controls, demonstrating the presence of a response mechanism capable of maintaining systemic T despite the loss of one testis. To our knowledge, this is the first study to characterize the rapid compensation of T secretion that occurs following hemi-castration in prepubertal lambs. Previous reports show that circulating T concentrations are similar between INT and HC sheep in samples collected at weekly or monthly intervals (Walton et al., 1978, 1980; Jenkins and Waites, 1983). In samples collected every other day, Johnson et al. (1971) found that T in HC rams recovered to INT concentrations by 8 d post-surgery. A rapid compensation in T production has also been documented in hemi-castrated rats (Frankel and Mock, 1982; Frankel and Wright, 1982; Mock and Frankel, 1982; Frankel et al., 1984) and bulls (Boockfor et al., 1983). In the present study, it is unlikely that significant testicular hypertrophy occurred by 1 wk after hemi-castration, therefore the recovery of serum T in HC rams suggests an increase in testicular T secretion by the remaining testis. In adult rats, stabilization of systemic
TABLE 2. MEAN (± SE) TESTICULAR GONADOTROPIN RECEPTORS IN INTACT (INT) AND HEMI-CASTRATED (HC) LAMBS

<table>
<thead>
<tr>
<th>Item</th>
<th>INT</th>
<th>HC</th>
</tr>
</thead>
<tbody>
<tr>
<td>LH receptors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>fmol/mg protein</td>
<td>3.8 ± 0.8</td>
<td>3.6 ± 0.5</td>
</tr>
<tr>
<td>pmol/testis</td>
<td>2.5 ± 0.3</td>
<td>3.8 ± 0.4*</td>
</tr>
<tr>
<td>$K_d \times 10^{-11}$ M</td>
<td></td>
<td>7.40 ± 0.01</td>
</tr>
<tr>
<td>FSH receptors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>fmol/mg protein</td>
<td>33.7 ± 3.2</td>
<td>33.2 ± 2.6</td>
</tr>
<tr>
<td>pmol/testis</td>
<td>22.9 ± 2.9</td>
<td>35.8 ± 2.7*</td>
</tr>
<tr>
<td>$K_d \times 10^{-10}$ M</td>
<td></td>
<td>2.23 ± 0.16</td>
</tr>
</tbody>
</table>

*aApparent dissociation constant for standard membrane pool. Receptor binding in unknowns and standard were parallel indicating receptors had similar affinities for the labeled ligand.

In sheep, Monet-Kuntz et al. (1984) reported that Sertoli cell division does not occur after 40 d of age, while Leydig cell numbers continue to increase through puberty. The increased content of FSH receptors in testes from HC rams probably reflects an increase in the number of binding sites per cell, while the increased LH receptor content is likely caused by an increase in both cell size and number. We did not detect any differences in the concentrations of LH or FSH receptors between INT and HC testes. Thus, the 40 to 50% increase in receptor content was due only to the overall increase in testis size in testes from HC rams, and does not fully explain the compensation in androgen secretion observed in these animals. In agreement with this study, Frankel et al. (1984) found no differences in LH receptor concentration or content 24 h following hemi-castration in adult rats. It is still possible, however, that dynamic short-term changes in testicular receptors may have occurred during the first few weeks following hemi-castration in these rams when serum FSH was elevated. Follicle stimulating hormone increases the numbers of LH and FSH receptors in the testes of several species (Ketelslegers et al., 1978; Tsutsui and Ishii, 1978).
et al., 1984), gilts (Redmer et al., 1984) and heifers (Johnson et al., 1985). However, in contrast to males, the elevation in FSH observed in females was sustained for less than 48 h following surgery. The increase in serum FSH following hemi-castration is probably due to removal of a gonadal inhibin source that preferentially suppresses its release (Franchimont et al., 1978). In males, the FSH-inhibiting factor is produced by Sertoli cells (Steinberger and Steinberger, 1976). It is now believed that the increase in FSH secretion following hemi-castration is, at least partly, responsible for gonadal hypertrophy (Cunningham et al., 1978; Walton et al., 1980; Jenkins and Waites, 1983; Waites et al., 1983). Concentrations of serum FSH in HC rams were elevated for 4 wk and then declined to pre-surgery concentrations at about the expected time of puberty. Similar pubertal decreases in serum FSH occur within 10 to 14 wk following hemi-castration of neonatal lambs (Walton et al., 1978, 1980; Waites et al., 1983).

Although sperm production per testis was increased in HC compared with INT rams, the amount of sperm produced per gram of tissue was unaltered. Increased spermatogenic yield has been reported in bulls (Boockfor et al., 1983) and rams (Voglmayr and Mattner, 1968; de Reviers et al., 1980; Hochereau-de Reviers et al., 1980); but, in most cases, the increase in sperm production by the HC testis was related more to an increase in testis size rather than an increase in spermatogenic efficiency. There are some notable exceptions, however. Hochereau-de Reviers et al. (1976) reported production of round spermatids/testis by adult rams hemi-castrated in the spring increased 70%, while testicular weight only increased 32% compared with INT rams. When rams were hemi-castrated in the fall, both sperm production and testis weight were increased 44 to 46%, supporting observations that season influences the hemi-castration response (Hochereau-de Reviers et al., 1976). In other studies, Johnson et al. (1971) observed a 53% increase in the spermatogenic efficiency of adult hemi-castrated rams; Johnson and Neaves (1981) observed similar increases in aged, but not young adult, male rats. Perhaps compromised testes with submaximal sperm production (e.g., aged animals or seasonal animals during the non-breeding season) are more capable of responding to hemi-castration, with increases in spermatogenic efficiency irrespective of increases in testicular size.

In summary, hemi-castration of prepubertal ram lambs altered the gonadal-hypophyseal-endocrine axis, increased FSH secretion and increased the weight of the remaining testis. Although HC rams possessed less total testicular tissue compared with INT rams, T production was similar throughout most of the study, without a detectable change in LH secretion. Although there were more LH and FSH receptors and sperm numbers in the larger remaining testis of HC rams, these differences were no longer apparent when data were expressed on a per-gm-tissue basis.

**Literature Cited**


