PRENATAL TESTOSTERONE PROGRAMMING: ONTOGENY OF CHANGES IN TESTIS OF FETAL AND PREPUBERTAL MALE SHEEP.

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INTRODUCTION

The non-natural exposure to testosterone (T) in intrauterine developing males either from pathological origin, like in males being exposed to T from maternal origin as may occur in pregnant women bearing PCOS (1), or due to experimental exposure to T in animal models of prenatal androgenization, is a condition not free of deleterious consequences, as in female offsprings.

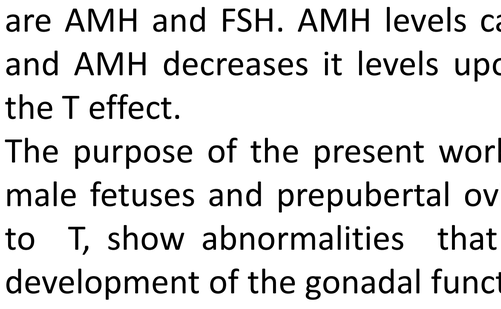
We have demonstrated that the prenatal exposure to T in ovine male causes a significant reduction in sperm count in adult rams. Moreover, we have observed a reduced sperm motility and a smaller scrotal circumference, suggesting a compromise in fertility (2). Another important feature in this animal model is a notorious increase in the number of Sertoli cells, which is not consistent with the reduced number of germ cells found in these animals, suggesting a decrease in the functionality of the Sertoli cells (2). At a molecular levels, these rams show an increased expression of the mRNA of the FSH receptor, which is important for Sertoli cells function and of some TGFbeta superfamiliy members involved in the blood-testis barrier (2). However the ontogeny of these alterations are not known.

Two of the key hormones in reproductive development are AMH and FSH. AMH levels can be modulated by FSH and AMH decreases it levels upon puberty onset due to the T effect.

The purpose of the present work was to inquire if ovine male fetuses and prepubertal ovine males, both exposed to T, show abnormalities that may predict an altered development of the gonadal function.

We studied the Sertoli and germ cells count and explored the expression of factors as AMH, AP2 (a transcription factor in part responsible of the enhancing effects of FSH upon AMH secretion), the AMH receptor II and intercelular communication factors in the seminiferous tubules such as N-cadherin and Conexin-43.

Protocol of T treatment in pregnant sheep (3)



Male fetuses at 120 days gestation

Male sheep at 24 w of age (prepubertal)

Histology: Tissues fixed in Bouin´s solution, later embedded in paraffin. Sections (3 µm) stained with H-E. Morphometry 5 sections/ animal were analyzed, each section was separated from the next by 400 µm. The quantification of the cell populations and the different seminiferous tubules parameters were identified according to the histological criteria used previously (Rojas-García et al., 2010, 2012).

Molecular Biology: Total RNA extraction and Reverse Transcription, before Real Time PCR performed in a Rotor-Gene RG-6000 thermocycler.The expression of Ubiquitin was chosen as the internal control in male fetuses and GAPDH in prepubertal male sheep. The results of real time PCR are presented as a ratio between the specific mRNA gene and the corresponding housekeeping gene.

Western Blotting: . Primary antibodies againts MISRII (Santa Cruz Biotechnology) diluted 1:1500 in TBS-Tween and anti N-cadherin and anti-. Seconday antibodies diluted 1:5000 (goat anti-rabbit POD, Amersham, or donkey anti-goat HRP, Santa Cruz Biotechnology, Inc.).

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RESULTS

Table 1. Body and testicular weight at prenatal and postnatal ages.

	C-males	T-males
Prenatal age: 120 days		
Body weight (Kg)	1.99 ± 0.06	2.3 ± 0.25
Testicular weight (g)	0.3357 ± 0.015	0.3170 ±0 0.049
Postnatal age: 24 weeks		
Body weight (Kg)	29.63 ± 1.85	24.66 ± 1.26 *
Testicular weight (g)	81.09 ± 10.11	52.47 ± 14.23 *

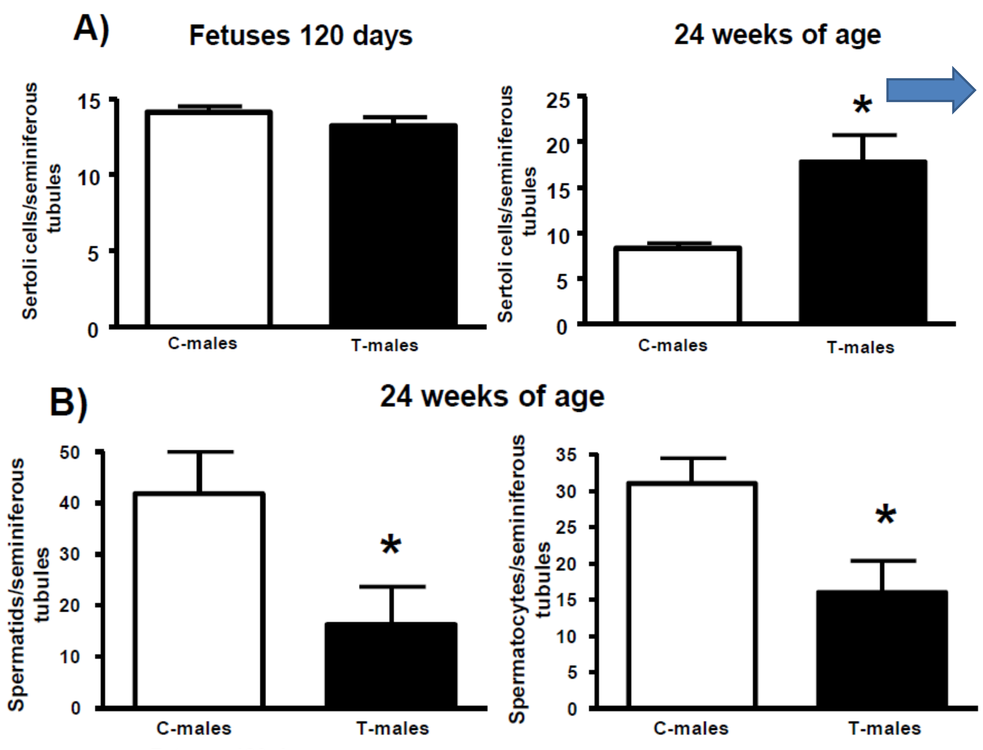

Figure 1. Morphometric counting of different cellular types. A number of 46 (C-males) and 49 tubules (T-males) were analysed. Sertoli cell number was significantly higher in T-males. (A). At 120 days of gestational age, SC number was equivalent in both groups. On the other hand, in prepubertal rams, germ cell number was lower in T-males, similar to what has been observed in adult rams (B).

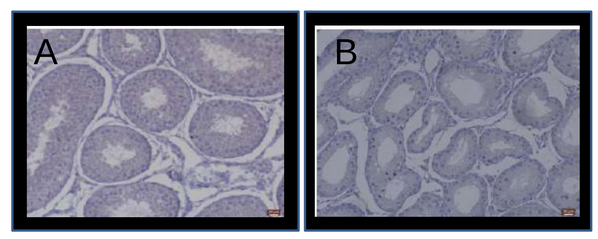

Figure 2. Photomicrography of prepubertal testicular section of a C-males (A) and a T-male (B). Size of the bar: 20 µm. A noticeable difference is observed, in which T-males show a remarkable decrease in tubule diameter and a loose intersticial tissue.

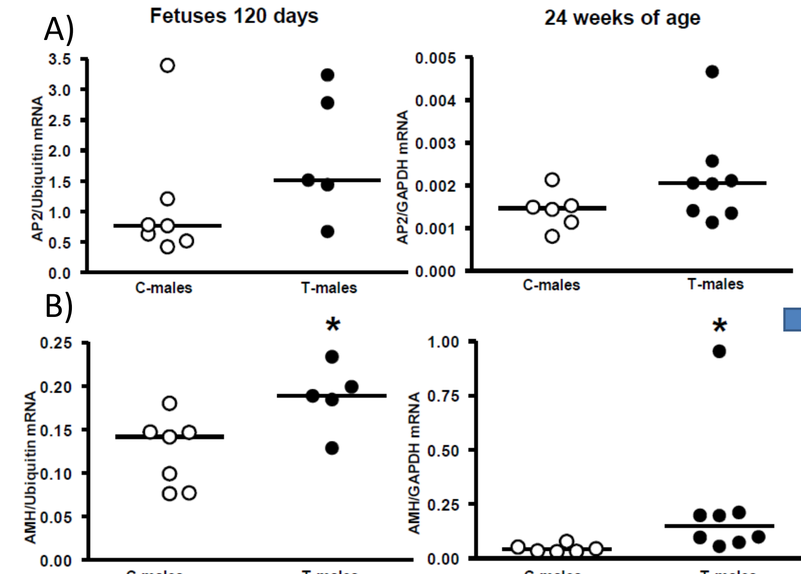

Figure 3. mRNA expression of AP2 (A) and AMH (B) in testicular tissue. AP2 expression did not show an overt difference between groups at both ages studied. AMH´s mRNA, however, was significantly highly expressed in T-males both at 120 day gestation and at 24 weeks.

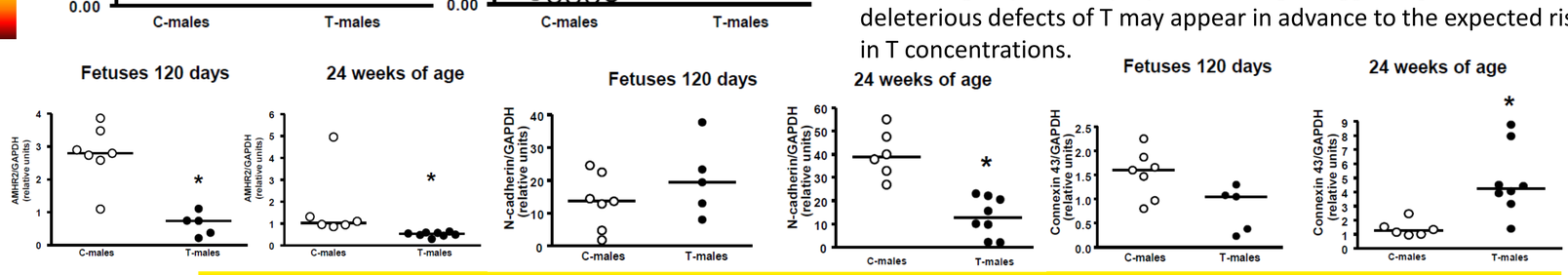

Figure 4. Western blotting of the AMH RII, N-cadherin and Connexin-43 in testicular tissue, showing the significant differences between groups at the prepubertal stage, suggesting that deleterious defects of T may appear in advance to the expected rise in T concentrations.

DISCUSSION

Abnormal cell count in the seminiferous tubule seems to be a defect appearing at prepubertal ages, not in fetal life, which may lead to impairment of normal spermatogenesis in adulthood. AMH expression showed an increase in T-males at both ages, which may allow to classify it as a permanent marker of the deleterious effect of excess T during gestation and suggesting a maintained immature status of that gonad, as well. The increase in AMH may not be explained by AP2, since this transcription factor showed similar expression between groups, suggesting the role of others factors not studied here. The immature gonad showed by the T-males, in times where maturation should be achieved, can also be presented as a dysfunctional blood testis barrier, (not properly attached cell-to-cell junction) which allows the passage of undeveloped germ cells to the seminiferous tubule lumen. The impact of prenatal T exposure can also be observed in testicular weight which suggest a not fully developed gonad. Therefore, alterations in testis of rams after prenatal exposure to T, can be regarded as a potential risk in their future fertility. These findings could be translated, with caution, to sons born to PCOS mothers.

References

[1]] Recabarren SE, Sir-Petermann T, Rios R, et al. J Clin Endocrinol Metab.93:3318-24, 2008.

[2] Rojas-García PP, Recabarren MP, Sarabia L, et al. Am J Physiol Endocrinol Metab. 299:E998-E1005, 2010.

[3]Recabarren SE, Rojas-García PP, Recabarren MP, et al. Endocrinology 149:6444-8 2008.