Lectin binding pattern of glycoconjugates during spontaneous testicular recrudescence in Syrian hamster (*Mesocricetus auratus)* after exposure to short photoperiod

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Running title: Lectin histochemistry in testicular recrudescence

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Summary

Lectin histochemistry was used to characterize glycoconjugates and cellular apoptosis in the seminiferous epithelium and interstitium of hamster testis during spontaneous recrudescence. An increase in the LTA lectin affinity was observed in spermatids in the Golgi phase. An increase in labelling of PNA and Con-A lectin in acrosome of spermatids (acrosome phase) as well increased labelling with Con-A in spermatids (cap phase) was observed. Spermatocytes showed decreased affinity with PNA and AAA lectins and an increase in positivity for LTA and GNA lectins. Spermatogonia showed a slight decrease in positivity to WGA and an increase in labelling with Con-A, and a decreased affinity for the AAA lectin. At the end of recrudescence all these germinal cells showed a similar pattern to the control. The Sertoli cells showed a gradual decrease in labelling with the GNA lectin and the Leydig cells an increase in labelling with Con-A and GNA. Particularly unusual was the observation of apoptotic spermatocytes and spermatids positive for PNA, GNA, AAA and Con-A, together with spermatocytes positive to LTA. In conclusion the normal lectin pattern is recovered during testis recrudescence and germ cell apoptotic activity is low, as is observed by specific lectins for germ cells in apoptosis.

Key words: Testes. Glycoconjugates. Lectins. Hamster. Apoptosis. Recrudescence.

1. INTRODUCTION

Changes in glycoprotein expression at the sperm surface have been observed during spermiogenesis, epididymal maturation, capacitation and the acrosome reaction in many mammalian species and also in different reproduction states (Nicolson et al., 1977; Schwarz & Koehler, 1979; Vázquez et al., 1993, 1996; Abd-Elmaksoud et al., 2008; Desantis et al., 2010; Toyonaga et al., 2011; Gomez-Torres et al., 2012; Parillo et al., 2012). The application of lectin histochemistry to testes has revealed a sequence of glycosylation processes during acrosome formation, and differential lectin binding patterns of Sertoli cells, Leydig cells and spermatogenic cells in the testes of many mammalian species (Arya & Vanha-Perttula, 1984, 1985a, 1985b, 1986; Malmi et al., 1990; Söderström et al., 1984; Kurohmaru et al., 1991, 1995, 1996; Jones et al., 1992a, 1992b, 1993; Martínez-Menárguez et al., 1992, 1993; Wine & Chapin, 1997; Verini-Supplizi et al., 2000; Calvo et al., 2000; Pinart et al.,2001a and 2001b; Desantis et al., 2006; Parillo et al., 2012), including humans (Malmi et al., 1987; Wollina et al., 1989; Arenas et al., 1998). Besides, the use of lectin histochemistry has been used in histopathological studies of testes (Pinart et al., 2001a; Gheri et al., 2004;Sato et al., 2012) and, recently, in our laboratory, for the apoptotic identification of germ cells in the seminiferous epithelium (Seco-Rovira et al. 2013, 2017).

In male hamsters, photoperiods involving less than 12 h of light per day induce a decrease in the weight of the testes, epididymis and accessory glands (Reiter, 1968). This testicular regression is initiated by a decrease in FSH, LH, testosterone and prolactin plasma levels (Berndtson & Desjardins, 1974; Kelly et al., 1994). Histologically, in Syrian hamster an involution of both seminiferous epithelium and interstitium has been observed in the regressed testes (Seco-Rovira et al. 2015, Beltran-Frutos et al., 2016a and 2016b). Tubular sections showed a seminiferous epithelium consisting of Sertoli cells, spermatogonia and only a few spermatocytes. During testicular regression, Sertoli cell death and a decrease in several

histomorphometric parameters have been observed (Seco-Rovira et al., 2015). At the cellular level, epithelium depletion is caused by an initial decrease in proliferation and increase in apoptosis involving all germ cells. At the end of the regression process, the proliferative and apoptotic activities of the spermatogonia recover the values observed prior to regression, probably in preparation for recrudescence (Morales et al., 2007; Seco-Rovira et al., 2015). In this recrudescence, an increase in proliferative activity and a decrease in apoptotic phenomena in germ cells, associated with the restoration of testosterone activity were observed (Martínez-Hernández, et al., 2018).

In Syrian hamster, which is widely used in reproduction studies, the lectin binding-pattern is already well known in both gonadally-active and regressed animals (Ballesta et al., 1991, Suda et al., 1998; Pastor et al., 2003). In regressed hamster, testes period with androgenic deprivation, changes in glycoconjugate patterns are reflected in the modification of N- and O-linked glycans expression and in their distribution patterns in both tubular and interstitial compartments (Pastor et al., 2003). However, changes in glycoconjugates due to testosterone restoration during the spontaneous testicular recrudescence after exposure to short photoperiod have not been described in the seminiferous epithelium or testicular interstitium_. The changes of glycoconjugates in relation to testosterone levels in a natural testosterone model could provide important information about reproductive biology, since glycoconjugates are involved in spermatozoa formation, maturation, capacitation and fertility (Liu, 2016).

As we previously found, lectin histochemistry is a suitable tool for identifying germinal cells in apoptosis, especially spermatocyte and round spermatids, undergoing apoptosis (Seco-Rovira et al., 2013, 2017). Apoptotic cells can be detected by several lectins that show an affinity for the glycoconjugate residues of Gal β1, 3-GalNAga1, α-D-mannose, N-acetylgalactosamine and L-fucose (Seco-Rovira et al., 2013).

Therefore, the aim of the present study was to examine the lectin-binding patterns in hamster testis during spontaneous recrudescence after exposure to short photoperiod. More specifically, the aim was to: a) analyze changes in glycan expression in the testicular interstitium and seminiferous epithelium during recrudescence to determine possible modifications in glycoprotein expression during androgenic restoration and b) analyze apoptosis in the seminiferous epithelium during recrudescence using lectin histochemistry.

2. MATERIAL AND METHODS

2.1 Reagents

Horseradish peroxidase (HRP)-conjugated lectins (PNA, , Con-A, SBA, HPA, LTA, UEA-I, WGA,), 3,3'-diaminobenzidine (DAB), bovine serum albumin (BSA), D-galactose, α-methyl-mannopyranoside, L-fucose, D-N-acetylglucosamine, D-N-acetylgalactosamine, and N-acetylneuraminic acid were purchased form Sigma (St. Louis MO, USA). Digoxigenin (DIG)-labeled lectins (MAA, GNA, AAA, SNA), and anti-DIG antibody were obtained from Boehringer (Barcelona, Spain). The common names, sugar specificity, and concentration of the lectins are shown in Table 1.

2.2 Animals and tissue processing

A total of 25 healthy sexually mature 6-month-old male hamsters *(Mesocricetus auratus*) were used. They were maintained in the animal facility of the University of Murcia at a temperature of 20 ºC, with food and water available *ad libitum*. Twenty animals were subjected to a short photoperiod (8 h light and 16 h dark), and the others (Control group, five animals) were always maintained with a 14-10 h light-dark cycle. At 16 (five animals), 19 (eight animals) and 21 (seven animals) weeks, individual animals were sacrificed by an overdose of CO_2 in a closed chamber and the testes were immediately removed. This study was performed according to the ethical and legal standards mentioned in Spanish Royal Decree RDL 53/2013 of 10th October concerning the protection of animals used for experimentation and other scientific proposes, and 2010/63/EU legislation on animal protection. The samples were fixed in Methacarn solution (Chloroform, Methanol and Acetic Acid 6:3:1) for 8 h at room temperature and were then processed and embedded in paraffin. Sections (5-μm thick) were stained with haematoxylin and eosin. Three recrudescence groups were established: initial recrudescence (IR), advanced recrudescence (AR) and total recrudescence (TR). These groups were established according to the time of exposure to the short photoperiod (IR: 16 weeks; AR: 19

weeks; TR: 21 weeks), the proportion of tubular seminiferous recovery; normal category (IR: 0% ± 0; AR: 14.3% ± 2.6; TR: 21.3% ± 2.4; Control: 44.8% ± 3.4), testicular volume (10^3) (IR: 0.59± 0.07 mm^3; AR: 1,44 ± 0,046 mm^3; TR: 1.73 ± 0.06 mm^3; Control: 2.06 ± 0.11 mm^3) and weight (IR: 0.61± 0.08 g; AR: 1.49 ± 0.04 g; TR: 1.79 ± 0.06 g; Control: 2.1 ± 0.11 g) (Martínez-Hernández et. al 2018).

2.3 Lectin histochemistry

Sections were deparaffinized and rehydrated through a series of decreasing ethanol concentrations. For HRP-conjugated lectins, after a brief rinse for 5 min in TBS (Tris buffered saline, pH 7.4), samples were incubated in a solution containing 0.3% H_2O_2 to block endogenous peroxidase activity. After 3 further rinses in TBS, sections were incubated with lectins (Sigma) at the appropriate dilution (Table 1) in a moist chamber at room temperature for 2 h. After washing in TBS, sections were immersed in TBS containing 0.05% DAB and 0.015% H_2O_2 to demonstrate peroxidase-containing sites. Sections were then counterstained whit Harris haematoxylin, dehydrated, cleared and mounted in DPX (Mixture of Distyrene, a Plasticizer and Xylene).

In the case of DIG-conjugated lectins, sections were blocked with 0.3% H_2O_2 and then incubated with 1% BSA in TBS for 10 min. DIG-conjugated lectins were applied at the appropriate dilution (Table 1) in a solution consisting of 1% BSA, 0.05% Tween 20, and 0.05% Triton X-100 in TBS for 1 h 30 min at room temp. After rinsing in TBS, samples were incubated with an HRP-labeled anti-DIG antibody, for 1 h, and diluted at 0.6 U/ml in TBS. Peroxidase activity was stained as described above. Sections were counterstained with Harris haematoxylin, dehydrated, and mounted in DPX. The staining intensity of the different lectins in germinal cells was subjectively evaluated by consensus of two independent observers, each with considerable experience, and classified into four categories: +++, very strong labelling; ++, strong labelling; +, moderate labelling; –, absence of labelling. This evaluation method is

similar to that used in classical qualitative lectin histochemistry studies both by our group and other authors (Calvo et al., 2000, Parrillo et al., 2012).

2.4 Controls

To assess the specificity of the lectin staining, the following controls were used: (1) preabsorption of the lectins with their corresponding inhibitory sugar at a concentration of 0.2 M. except for those using a different concentration, as specified in Table 1; and (2) substitution of conjugated lectin by TBS to determine the presence of endogenous peroxidase activity.

3. RESULTS

3.1 Changes in glycoconjugate pattern during recrudescence

The lectin patterns obtained in the seminiferous epithelium (germinal cells and Sertoli cells) and in the interstitium (Leydig cells) of control animals were similar to the patterns described in our previous study (Pastor et al. 2003). During recrudescence, the Golgi in the spermatids in Golgi phase showed an increase in affinity with the LTA lectin until they reached (in the TR group) intensity similar to that of the control. The acrosome of the spermatids in the cap phase showed an increase in labelling with Con-A, before reaching levels that were similar to the control in the TR group. Finally, the acrosome of the spermatids in the acrosome phase showed an increase in affinity with the PNA and Con-A lectins until the intensity of the controls was reached in the TR group (Table 2).

The spermatocytes showed a decrease in labelling with the PNA and AAA lectins during the process of testicular recrudescence, while their positivity for LTA (Fig 1a-c) and GNA lectins increased until the pattern in TR was similar to that of the controls.

With Con-A, the spermatogonia (mainly type B) showed increased affinity during recrudescence. In addition, there was a slight decrease in WGA positivity, whereas the AAA lectin showed a decrease in positivity during testicular recrudescence until a pattern similar to the control was observed in the TR group (Fig 1 d-f).

The Sertoli cells showed a gradual decrease in GNA affinity during recrudescence (Fig 1 g-i). The Leydig cells showed, an increase in labelling with Con-A (Fig 1 j-l) and with GNA, whose positivity was very strong in the IR group, while a slight decrease in positivity was observed in the TR group compared to the AR group. Finally, no variation in the lamina propria pattern of glycoconjugates was observed during the process of testicular recrudescence with respect to the control group. Table 2 summarizes the results and changes found.

3.2 Detection of apoptosis during the process of testicular recrudescence through the use of lectins.

Of the battery of lectins used to characterize the glycoconjugate pattern in the seminiferous epithelium, PNA, GNA, AAA, Con-A and LTA showed strong positivity in the few cells that presented morphological characteristics corresponding to cells in apoptosis (condensation or nuclear fragmentation and separation of neighbouring cells).

With PNA, round spermatids were strongly positive to this lectin in the cytoplasm during recrudescence, especially in the IR group (Fig 2a and 2c). In addition, in this stage, tubular sections with large numbers of round spermatids strongly marked with PNA and tubular sections with almost non-existent labelling by this lectin were observed (Fig 2b). In more advanced stages of recrudescence, this positivity decreased until it was similar to the control group. More frequently in IR and less frequently in AR and TR, the PNA lectin strongly marked the cytoplasm of spermatocytes in pachytene, both in the initial stages and more advanced stages of apoptosis (Fig 2d).

GNA occasionally provoked strong marking in round spermatids at the cytoplasmic level (Fig 2e) mainly in cells that showed apoptotic alterations compared with others with normal morphological characteristics during the initial process of testicular recrudescence but hardly at all in the TR group. The spermatocytes showed strong labelling by GNA when their morphological characteristics were comparable to those of cells in a state of advanced apoptosis (Fig 2f), a fact observed occasionally in AR and TR groups.

With the AAA lectin, round spermatids were revealed from initial to advanced stages of apoptosis due to the strong positivity shown in the cytoplasm, especially in the IR group. The spermatocytes, from the initial to advanced stages of apoptosis, showed an increase in cytoplasmic positivity with AAA but not in very advanced stages of the apoptotic process. Such positivity was also observed in the AR and TR sections, but to a much lower degree.

In the case of Con-A, the altered round spermatids showed a more intense affinity at cytoplasmic level than normal ones during the process of testicular recrudescence, and were observed from the early stages of their entry into apoptosis in the IR group. On the other hand, spermatocytes with typical characteristics of cells in apoptosis appeared strongly marked by this lectin mainly at the cytoplasmic level, although in more advanced stages of apoptosis, positivity was also evident at the nuclear level. This was observed sporadically in the three recrudescence groups. Finally, the LTA lectin showed strong positivity in spermatocytes in the initial stages of apoptosis, which was maintained in different apoptotic stages (Fig 2g), although the spermatocytes positive for this lectin decreased as recrudescence progressed until they were very scarce in the TR group (Fig 2h).

4. DISCUSSION

During the spontaneous testicular recrudescence that occurs after continuous exposure to a short photoperiod, the lectin binding pattern that exists in the reproductive conditions associated with a long photoperiod is gradually re-established. This recovery affects all germ cell populations, some increasing their positivity to certain lectins and others decreasing their positivity, although a quantitative assessment was not made. It is well known that lectins are useful tools for studying the distribution patterns of cellular glycoconjugates (Spicer, 1993) and the changes that take place during the differentiation and maturation of germinal cells in the seminiferous epithelium (Malmi et al., 1990). The results obtained for the spermatids are probably related to the formation and maturation of the acrosome, as well as to the disappearance of spermatids undergoing apoptosis. It is known that the acrosome presents O-linked glycoconjugates and N-linked glycoconjugates (Martínez-Menárguez et al., 1992). Con-A has an affinity for the mannose, acarbohydrate-residues, that form the initial portion of the N-glycoconjugate chains. The increase in its affinity in the cap and acrosomal spermatids during recrudescence would indicate that the content of these glycoconjugates gradually reaches an appropriate concentration in the acrosomes. The strong affinity of round spermatids toward PNA that was observed is associated with portions of the seminiferous tubules that suffered spermatogenesis arrest during testicular regression due to short photoperiod, in which there is a strong apoptosis of these cells (Seco-Rovira et al., 2015). The gradual weakening of this strong positivity would therefore indicate a decrease in apoptosis in these cells during recrudescence. In IR there are still portions of the seminiferous tubule in spermatid arrest and with germ cells in apoptosis, together with portions in which, although apparently arrested, there is no mass apoptosis of these cells, indicating that these are areas where spermiogenesis is recovering. These results mean that PNA lectin is a good marker of the state of the seminiferous epithelium during recrudescence. In a previous study in gonadally-inactive hamsters (Pastor et al., 2003),

spermatocytes did not show staining with Con-A, GNA or LTA lectins, whereas the plasma membranes of these germ cells had a strong affinity for HPA, PNA and AAA lectins, suggesting loss of mannose and α-L-fucose residues and the appearance of α-D-N-acetylgalactosamine, α-D-galactosamine or β-D-galactosamine-β(1→3)-N-acetylgalactosamine, as well as an increase in α(1→6)-fucose residues on the cell surface, during regression. Proteins with affinity for β-galactoside residues, such as those of the galectin family, seem to be integral parts of the regulation process of cell growth, tissue modeling/remodeling, apoptosis and cell adhesion (Kaltner & Stierstorfer, 1998). Moreover, multiple fucosyltransferases and their carbohydrate ligands, and α-mannoses are involved in spermatogenic cell-Sertoli cell adhesion (Raychoudhury & Millette, 1997). Consequently, the inverse changes observed in glycoconjugate expression patterns in our study during the recrudescence of seminiferous epithelium appear to be related with a possible decrease in apoptosis of spermatocytes, as well with an end to the disruption of spermatogenic cell-Sertoli cell adhesion during the renovation of epithelium.

Spermatogonia in gonadally inactive hamsters showed an increase in affinity for AAA and a decrease in positivity for Con-A and GNA (Pastor et al., 2003). The results obtained in this study showed that the spermatogonia recover their normal pattern of affinity during recrudescence. As occurs in spermatocyte, these changes are very probably related with changes in germ cell-Sertoli cell interactions.

In gonadally inactive hamsters the increase of GNA affinity in Sertoli cells was observed in a previous study (Pastor et al., 2003) and may correspond to lysosomes resulting from phagocytosed residual bodies from degenerated germ cells after exposure to a short photoperiod. This process includes germ cell apoptosis and the subsequent phagocytosis of degenerated germ cells and spermatids by Sertoli cells (Seco Rovira et al., 2014). During recrudescence, the decrease in GNA affinity probably indicates lower lysosomal activity and a

decrease in the residual bodies in the Sertoli cells, which are very abundant during testicular regression.

Leydig cells of gonadally active hamsters contained glycans with fucosyl, mannosyl, glucosyl, neuraminic acid and galactosyl residues, which have structural and transport functions and participate in androgen synthesis and cell regulation (Pinart et al., 2002). The strong staining with Con-A and GNA lectins (specific for α-mannose residues of N-linked oligosaccharides) may be related to regulation of luteinizing hormone (LH)-receptor in Leydig cells (Arenas et al., 1998). N-linked carbohydrate chains of the rat ovarian LH receptor have been shown to be essential for high-affinity hormone binding (Zhang et al., 1995). It has been suggested that LH/hCG receptors are sialoglycoproteins with predominantly N-linked glycosyl residues, which would account for the size differences between testicular and ovarian receptors, and they may participate in interactions with gonadotropin (Minegishi et al., 1989). Moreover a lack of glycosylation decreases LH receptor processing, leading the receptors for degradation and thereby to decrease its presence in cell membrane (Clouser & Menon, 2005). The increased expression of N-linked carbohydrate chain residues observed in hamster Leydig cells in recrudescence may reflect an increase in their affinity for LH and accordingly the recuperation of androgen production that occurs in the animals during recrudescence**.**

In the recrudescence groups studied some cells, especially spermatocytes and round spermatids, were observed to be very positive towards PNA, GNA, AAA, Con-A and LTA lectins and showed clear signs of apoptosis. The results coincide with that observed in a previous study concerning the use of lectins as a tool for identifying germ cells in apoptosis (Seco-Rovira et al., 2013). The main difference is that during the testicular regression due to short photoperiod, the number of spermatocytes and spermatids is abundant compared with those in the non-photoinhibited testes, making identification with those lectins, easier (Seco-Rovira et al., 2013). The results of our study show that the hormonal restoration that occurs

during recrudescence is accompanied by a decrease in apoptotic activity that will probably help the recovery of normality in spermatogenesis in the seminiferous epithelium. Likewise, they indicate that, even though these cells are scarce, they show an affinity pattern for lectins similar to that seen when they are abundant in the regressed animals, which means that glycoconjugate changes are specific to the apoptotic process in germ cells. In this respect, it should be noted that the spermatids that show low apoptosis in the testes during long photoperiod and massive apoptosis during regression (Seco-Rovira et al., 2015) undergo an inverse process during recrudescence, as is shown by their affinity for the PNA lectin, which is very specific for spermatids in apoptosis and whose affinity disappears abruptly in spermatids during recrudescence.

5 CONCLUSION

Recrudescence involves changes in the expression of glycans in the testicular seminiferous epithelium while the interstitium recovers its normal lectin pattern. The low apoptotic activity shown by germinal cells during recrudescence is revealed by the same lectins that did so during the regression phase, when the same cells showed a high degree of apoptosis. These modifications in glycoprotein expression are probably due to the androgenic restoration that occurs during recrudescence and which leads to a recovery of normal spermatogenesis in testes.

ACKNOWLEDGEMENTS.

We thank M.C. Gonzalez Ulloa for her laboratory assistance (University of Murcia). The study was supported by grants Funded GERM 19892/15 from the Fundación Séneca, Comunidad Autónoma de la Región de Murcia.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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Tables.

Taxonomic name	Acronym	Concentration	Specificity	Inhibitory sugar
Galanthus nivalis	GNA	10 μm ml^{-1}	Man α1,3-Man-	Methyl -α-Man
Canavalia ensiformis	Con-A	15 μm ml^{-1}	α-D-Man > α-D-Glc	Methyl-α-Man
Glycine max	SBA	12 μm ml^{-1}	α-D-GalNAc > β-D-Gal	NAc D-Gal NAc
Helix pomatia	HPA	12 μm ml^{-1}	α-D-Ga NAc α1,3-Gal	NAc D-Gal NAc
Triticum vulgaris	WGA	6 μm ml^{-1}	Gal β1,4-GlcNAc β1 > GlcNAc β1 > Neu5Ac	D-Glc NAc (0.7 M)
Arachis hypogea	PNA	12 μm ml^{-1}	Gal β1,3-GalNAcα1	β-D-Gal (0.4 M)
Ulex europaeus	UEA-I	25 μm ml^{-1}	L- Fuc α1,2-Gal β1,4-GlcNAc β1-	L-Fuc
Aleuria aurantia	AAA	15 μm ml^{-1}	L-Fuc α1-6-	L-Fuc
Lotus tetragonolobus	LTA	25 μm ml^{-1}	α-L-Fuc	L-Fuc
Maackia amurensis	MAA	15 μm ml^{-1}	Neu5Ac α2,3-Gal β1,4-Glc NAc β1	Neuraminic acid lactose
Sambucus nigra	SNA	15 μm ml^{-1}	Neu5Ac α2,6-Gal/Gal NAc	Neuraminic acid lactose

Table 1: Concentration, specificity and inhibitory sugars of lectins used in the present study.Abbreviations: Fuc, fucose; Gal, galactose; Gal NAc, N-acetyl-galactosamine; Glc, glucose; Glc NAc, N-acetyl-glucosamine; Man, mannose; Methyl-α-Man, methyl alpha mannose; Neu5Ac, Neuraminic acid.

Cell Type	Lectin	IR	AR	TR	Control	Phase*
Spermatids	LTA	-	+	++	++	Golgi
	Con-A	+	+/++	++	++	Cap
	PNA	+	++	+++	+++	Acrosomal
	Con-A	+	++	+++	+++	
Spermatocytes	AAA	++	+	-	-	
	PNA	++	+	-	-	
	LTA	+	++	+++	+++	
	GNA	-	+	++	++	
Spermatogonia	Con-A	+	++	+++	+++	
	WGA	+	+	-	-	
	AAA	+++	++	+	+	
Sertoli cells	GNA	+++	++	+	+	
Leydig cells	Con-A	+	++	+++	+++	
	GNA	+++	+++	++	++	

Table 2. Changes in lectin pattern during testicular recrudescence in cells of the seminiferous epithelium and interstitium. Abbreviations of staining intensity: +++, very strong labelling; ++, strong labelling; +, moderate labelling; +/++, moderate labelling with some strong cases, –, absence of labelling. * The lectin affinity was evident in the Golgi and acrosome organelles. The rest of the lectins showed a similar affinity pattern to the controls during recrudescence.

Figures

Fig 1. Changes in lectin pattern during testicular recrudescence. **a-c)** The spermatocytes, despite being stained with LTA lectin in IR (arrows) (a), showed an increase in positivity for this lectin during recrudescence (b) until they showed the same pattern as the control group (c). **(d-f)** The spermatogonia decreased their positivity for AAA lectin during recrudescence (d and e) until they showed a similar pattern to the control group (arrows) (f). **(g-i)** The Sertoli cells (arrows) showed a decrease in positivity for GNA lectin during recrudescence (g and h) until they showed a similar pattern to the control group (i). **(j-l)** The Leydig cells (arrows) increased their positivity for the Con-A lectin during recrudescence (j and k) until they reached a similar pattern to that of the control group (l). Real magnification 400x.

Fig 2. a-d) PNA lectin histochemistry. In **a)** a tubular section with very positive staining round spermatids (black asterisk) and a tubular section with no positivity of round spermatids (white asterisk) in the IR group. The upper insert shows some round spermatids with typical positivity for PNA lectin while the lower insert shows the strong positivity for PNA lectin in the round spermatids. **b)** In the AR group, the round spermatids in almost every tubular section show a similar staining pattern to the controls. **c)** An apoptotic round spermatid strongly stained with PNA lectin (arrow) and some normal round spermatids (arrowhead). **d)** An apoptotic spermatocyte with the cytoplasm very strongly stained with PNA lectin (arrow) compared with normal spermatocytes (arrowheads). **e-f)** GNA lectin histochemistry. In **e)** an apoptotic round spermatid strongly stained at cytoplasm level (arrow) and some normal round spermatids (arrowheads). In **f)** an apoptotic spermatocyte strongly stained (arrow) compared with some normal spermatocytes (arrowheads). **g-h)** LTA lectin histochemistry. **g)** In the IR group many apoptotic spermatocytes with strong staining at the cytoplasmic (arrows) level in the same tubular section compared with normal spermatocyte (arrowhead) can be observed. **h)** In the TR group the spermatids show a strong staining (arrows) similar to the control group but different from that shown by apoptotic cells observed in e). Real magnification a) and b) 200x; c-h) and inserts 400x.