Reproduction in Domestic Animals

Reprod Dom Anim doi: 10.1111/rda.12174
ISSN 0936–6768

Lectin-Binding Pattern in Ovarian Structures of Rats with Experimental Polycystic Ovaries

CG Barbeito[1,2], HH Ortega[2,3], V Matiller[3], EJ Gimeno[2,4] and NR Salvetti[2,3]

[1]*Cátedra de Histología y Embriología, Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, Esperanza, Santa Fe, Argentina;* [2]*Concejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina;* [3]*Cátedra de Biología Celular, Facultad de Ciencias Veterinarias, Universidad Nacional del Litoral, Esperanza, Santa Fe, Argentina;* [4]*Instituto de Patología, Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, Esperanza, Santa Fe, Argentina*

Contents
Numerous experimental models in different species have been developed for the study of polycystic ovarian syndrome. In this study, we used a model of induction of polycystic ovaries (PO) in rats by exposure to constant light to study the distribution and variations of glycosylated residues present in the different ovarian structures. Seven biotinylated lectins were used (Con-A, WGA, DBA, SBA, PNA, RCA and UEA-I) on tissue sections, and detection was performed using the streptavidin/peroxidase method. In tissue sections was observed an increase in affinity for Con-A in the granulosa and theca interna of growing follicles and cysts in animals with PO in relation to the control group. Follicular cysts showed higher affinity for WGA and RCA-I which growing follicles in the same group, and there was a decrease in affinity for PNA in the cysts in relation to the growth of follicles in both groups. Atretic follicles in both groups showed greater labelling with lectins PNA, SBA and RCA-I in relation to healthy follicles. It could also be noted that the zona pellucida of cystic follicles lost the affinity for the lectin Con-A. There was no staining on follicles in any category with the lectins DBA and UEA-I, although it was staining in the corpus luteum (control group) and in the mesothelium and interstitial glands of both groups with DBA. These observations probably reflect changes in the glycosaminoglycans present in the different ovarian compartments or in the glycosylation of cellular components essential for proper follicular dynamics.

Introduction

Ovarian cysts are persistent anovulatory follicular structures in the absence of a corpus luteum, which spontaneously occur in several species for example cattle, sheep, goats, pigs and even human beings (Vanholder et al. 2006; Regassa et al. 2009; Pauli et al. 2011; Sun et al. 2011). Many factors are associated with the development of ovarian follicular cysts, although their precise relationship remains unclear (Silvia et al. 2002, 2005; Vanholder et al. 2006; Salvetti et al. 2010).

Since the 1960s, a range of animal models, including rodents, sheep and non-human primates, have been used to study the origins and pathology of polycystic ovaries (PO; Edwards 1971; Abbott et al. 1998; Singh 2005). These models have advanced our understanding of the pathogenesis of PO; however, at present, a convincing whole animal model representing all features associated with human polycystic ovarian syndrome (PCOS) has not been established (Walters et al. 2012). A range of characteristics similar to those seen in women with PCOS have been described in distinct animal models.

Numerous experimental models for PO have been developed in rats. The following hormonal substances, among others, have been employed to induce this syndrome: estradiol valerate, dehydroepiandrosterone and neonatal androgenization (Mahesh et al. 1987; Mahajan 1988). In spite of their effectiveness, all these hormonal treatments cause a sudden appearance of PO due to disturbances in the metabolic and physiologic processes. Thus, these models do not reproduce faithfully what happens in the real syndrome. The continuous administration of estrogens, like estradiol valerate, induces a progressive degeneration of the arcuate hypothalamic nucleus, which impairs the study of the hypothalamo-pituitary system (Mahajan 1988). The treatment with androgens can interfere with luteinizing hormone (LH) release in the central nervous system. Therefore, this model may introduce variables not present in the spontaneous disease (Kalra and Kalra 1983; Mahesh et al. 1987). In fact, androgenized rats showed morphologic and endocrine patterns, which are not completely correlated with those of the PCOS, and consequently, androgenization could not be an appropriate model for the study of this syndrome (Jones et al. 1987).

Exposing to constant light is a simple method to induce PO in cycling rats. In environment with constant illumination, the hypothalamus fails to induce pre-ovulatory LH surge and ovulation is blocked, so the pre-ovulatory follicles persist and interrupt the normal oestral cycles (Salvetti et al. 2009a,b). The morphology of the ovaries resembles idiopathic PCOS with a thick tunica albuginea, numerous atretic follicles and cystic follicles in the absence of active corpora lutea (Singh 1969; Salvetti et al. 2004a). This model may provide a useful tool to test therapeutic and preventive management for a serious reproductive disease in many animal species, including livestock (Mahesh et al. 1987; Mahajan 1988; Baldissera et al. 1991; Szukiewicz and Uilenbroek 1998; Salvetti et al. 2004a).

Glycobiological investigations of reproductive biology in mammals suggest that oligosaccharides act as essential functional components of glycoproteins involved in fundamental steps, such as oocyte maturation (Eriksen et al. 1999; El-Mestrah and Kan 2001; Rath et al. 2005), sperm–oocyte binding and fertilization (Desantis et al. 2009).

The zona pellucida (ZP) is essential at the time of fertilization, and their composition varies among species. Zona pellucida consists of three to four major acidic glycoproteins (Grootenhuis et al. 1996; Parillo et al.

© 2013 Blackwell Verlag GmbH

2005). The protein components of these glycoproteins are highly homologous among species; conversely, the carbohydrate contents of the N- and O-linked oligosaccharides show interspecific differences, and these may relate to species-specific binding of sperm to egg (Chapman et al. 2000; Parillo et al. 2005). L-fucose has been shown to be involved in sperm–ZP recognition in guinea pig, hamster, rat and human oocytes (Chapman et al. 2000; Parillo et al. 2005), while in pigs, an N-linked glycans of ZP have a significant role in sperm–egg interaction (Yonezawa et al. 1997; Parillo et al. 2005). These studies provide strong evidence that the glycan portion of ZP glycoproteins is the ligand for spermatozoa.

On the other hand, glycosaminoglycans (GAGs) are a group of glycoconjugates with important roles in ovarian physiology (McArthur et al. 2000; Rodgers et al. 2003) that have been identified in the ovarian tissues and follicular fluid of pigs (Yanagishita et al. 1979), cows, (Bellin et al. 1983; Grimek et al. 1984), humans (Eriksen et al. 1999) and rats (Mueller et al. 1978). Proteoglycans are macromolecules formed by a core protein with one or more GAGs side chains covalently attached. They can be secreted by the cells, retained at the cell surface or stored in intracellular vacuoles. Hyaluronan is a long GAG, which is released into the extracellular matrix as a free polysaccharide not covalently linked to a protein. Both proteoglycans and hyaluronan influence many aspects of cell behaviour by multiple interactions with other molecules. They are involved in matrix formation, cell–cell and cell–matrix adhesion, cell proliferation and migration, and osmotic pressure maintenance and show coreceptor activity for growth factors. Proteoglycan and hyaluronan synthesis change significantly during ovarian follicle development and atresia (Salustri et al. 1999).

Lectin histochemistry has been successfully used to characterize *in situ* carbohydrates of ovarian follicles in numerous species (Maymon et al. 1994; Salvetti et al. 2000; Desantis et al. 2009). Lectins are a group of proteins which have the property of binding to certain specific sugar chain structures including those of glycoproteins (Debray et al. 1981), glycolipids (Zurn 1982) and GAGs (Toda et al. 1981; Bell and Skerrow 1984; Hirabayashi 2008; Desantis et al. 2009).

In previous works, we have demonstrated changes in the expression of extracellular matrix components (Salvetti et al. 2003), cytoskeleton proteins (Salvetti et al. 2004b), steroid receptors (Salvetti et al. 2009a), proliferation/apoptosis balance (Salvetti et al. 2009b) and growth factors (Ortega et al. 2007) in the polycystic ovary. However, the normal pattern and possible changes of the carbohydrates in the glycoconjugates of the different structures in the cystic ovaries have not been reported. The aim of this work was to analyse the saccharides of the glycoconjugates in normal ovaries and induced PO using lectin histochemistry technique.

Materials and Methods

Animals and treatment

All procedures were carried out according to the Guide for the Care and Use of Laboratory Animals (NRC, 1996). Female Wistar rats (16 weeks old) were provided by Centro de Experimentaciones Biológicas y Bioterio (Facultad de Ciencias Veterinarias, Universidad Nacional del Litoral). Before the experiment, the animals were kept under a controlled cycle of light–darkness (lights on from 8:00 h to 20:00 h), at 18–22°C with free access to water and commercial food (Cargill, Argentina). Lighting was provided by banks of General Electric 4 cool white 40 W fluorescent tubes to obtain an intensity of 350 lx to 1 m from the floor.

Fifteen animals displaying at least two normal 5-day oestrous cycles just prior to treatments were divided in two groups; the continuous light group (n = 10) was placed in the conditions described except that the light cycle was extended to 24 h. A control group (n = 5) of females of the same age as the treated animals remained in the normal light–dark conditions (Salvetti et al. 2004a).

Smears obtained by vaginal washing were examined under a microscope for the relative abundance of nucleated epithelial cells, cornified cells and leucocytes. Cycles with duration of 5 days were considered regular. The presence of cornified cells in the smears for a minimum of 10 consecutive days was defined as persistent vaginal cornification and considered as confirmation of follicular cystic development (Salvetti et al. 2004a).

Tissue sampling

The animals of light-exposed group were sacrificed 2 weeks after that persistent vaginal cornification was established. The animals of control group were sacrificed after 10 weeks, in pro-oestrus to obtain pre-ovulatory tertiary follicles.

After being anaesthetized with a cocktail of ketamine/xylazine (40/4 mg/kg) via subcutaneous injection, the rats were killed by decapitation. The ovaries were dissected and fixed in 10% (v/v) buffered formalin for 6 h at 8°C and were washed in phosphate-buffered saline (PBS). For light microscopy, fixed tissues were dehydrated in an ascending series of ethanol, cleared in xylene and embedded in paraffin. Five-micrometre-thick sections were mounted in slides previously treated with 3-aminopropyltriethoxysilane (Sigma-Aldrich, St. Louis, MO, USA) and were stained with haematoxylin–eosin for a previous observation.

Lectin histochemistry

Ovarian sections of all animals were deparaffinized, hydrated and incubated in 0.3% hydrogen peroxide in methanol for 30 min at room temperature, rinsed several times in 0.01 M PBS, pH 7.2, and treated with 0.1% bovine serum albumin in PBS for 15 min. Then, they were incubated with the biotinylated lectins (Lectin Biotinylated BK 1000 Kit; Vector Laboratories Inc., Burlingame, CA, USA) overnight at 4–8°C. Table 1 shows the specificity of the used lectins.

The optimal concentration for each lectin, which allowed maximum staining with minimum background, was at a dilution of 10 μg/ml in phosphate-buffered saline (PBS). The incubation was performed all night at

© 2013 Blackwell Verlag GmbH

Table 1. Lectins used in this study and their major specificities

Acronym	Source	Major specificity
UEA-1	*Ulex europaeus*-1, *Gorse*	α1,2-linked fucosyl residues
DBA	*Dolichos biflorus, Horse gram*	GalNAcα1,3(LFucα1,2)Galβ1,3/4GlcNAcβ1
PNA	*Arachis hypogaea, Peanut*	Galβ1,3GalNAcβ1- >Galβ1,4GlcNAcβ1-
SBA	*Glycine max, Soybean Terminal*	GalNAcα1- >Galα1
WGA	*Triticum vulgaris, Wheat germ*	Di-*N*-acetyl chitobiose, *N*-acetyl lactosamine and some sialyl residues
RCA-1	*Ricinus communis*	ß-D-galactose
Con-A	*Concanavalia ensiformis*	α-D-glucose and α-D-mannose

4°C. Detection was by a streptavidin–peroxidase solution (BioGenex, San Ramon, CA, USA) with 3.3-diaminobenzidine (DAB; Dako, Carpinteria, CA, USA) as chromogen. All sections were counterstained with activated haematoxylin (Biopur, Rosario, Argentina).

The following controls were performed: the lectins were omitted or blocked by incubating them with their blocking sugars (0.1–0.2 M in PBS) for 1 h at room temperature before application to the sections.

Analysis

Each lectin was analysed in five sections (minimum) of each ovary. The lectin binding was analysed using the following semiquantitative scale of the stained structures and subjectively scored as follows: (−) none, (+) weakly positive, (++) moderate positive and (+++) strongly positive. We evaluated the following structures: granulosa, theca interna, ZP and oocyte in growing normal (both groups) and cystic follicles (PO group); granulosa in atretic follicles, interstitial glands, mesothelium and endothelium of blood vessels in both groups and corpus luteum in control group.

Results

Lectin-binding pattern

In control group, we observed primordial, growing primary, secondary and tertiary follicles, atretic follicles, as well as interstitial glands. In constant light-exposed animals, we found follicles at different stages of development, in addition to follicles with evidence of atresia, and many large cysts with thickened granulosa cell layer. Corpus luteum was absent, and hyperplasia of interstitial glands was also observed.

The Table 2 summarized the lectin histochemical staining.

It was observed an increment in the affinity with Con-A in the granulosa and theca interna of the cysts and growing follicles as the granulosa of atretic follicles in the ovaries of PO group in relation to the structures in the control group. The ZP of the cysts did not present staining, although the growing follicles show an increment when compared to growing follicles of the control group and either staining was evident in oocytes. Interstitial glands and mesothelium show moderate staining in both groups, while the endothelium of vessels of both groups and corpora lutea show weakly positive staining (Fig. 1).

With PNA was observed a diminution in the affinity in granulosa cell layer of cysts in relation to growing follicles of both groups; however, an increment in the intensity was observed in the control group. Oocytes of the cysts showed a minor intensity in the staining in relation to growing follicles. The ZP showed an intense staining, while no staining was detected in the theca interna for cysts or growing follicles. The interstitial glands showed moderate intensity of staining in some areas being higher in PO than in control group. Mesothelium and endothelium of vessels showed none or weak staining in both groups. A moderate staining was observed in corpora lutea (Fig. 1).

It was observed an increase in the reactivity with SBA in the theca interna and ZP of cysts in relation to growing follicles in both groups, without differences in the granulosa. The oocytes of the cysts showed a decrease in the staining. There was an increment in reactivity in the granulosa of atretic follicles as well as in the endothelium of blood vessels and was a decrease in the mesothelium and interstitial glands (Fig. 1).

With WGA was observed a decrease in the intensity in growing follicles in PO group in relation to the control group, although these structures showed no difference in the cysts. Both the ZP and the oocyte of the cysts showed a decrease in staining in relation to the growing follicles. The granulosa of atretic follicles, interstitial glands and endothelium of blood vessel showed a moderate intensity, while the mesothelium was observed strongly labelled in both groups (Fig. 1).

RCA-I showed intense reactivity in granulosa cells of the cysts and between zero and moderate in the same cells of growing follicles of both groups. The other components of cystic follicles showed no differences with the control group. In addition, there was a decrease in the intensity staining in the endothelium of blood vessels and mesothelium of PO group in relation to the control group (Fig. 2).

No reaction was seen with DBA and UEA-1 in any component of cystic or growing follicles. With DBA was observed an increment in the staining in the interstitial gland and a decrease in the mesothelium in the PO group. The corpora lutea showed a moderate intensity of staining. On the other hand, with UEA-1, only slight staining was observed in the mesothelium of the ovaries from PO group and moderate in the control group (Fig. 2).

Discussion and Conclusions

In the present study, we investigated the staining pattern of lectin-binding glycoconjugates in the ovaries of rats with PO in relation to control animals. We observe a

© 2013 Blackwell Verlag GmbH

Table 2. Lectin-binding pattern of different structures of the ovary in light-exposed and pro-oestrous control rats

	Con-A	PNA	SBA	WGA	RCA-I	DBA	UEA-I
PO group							
Cysts							
Granulosa	++/+++	–	+	+++	++/+++	–	–
Theca interna	+/++	–	++	+++	++	–	–
Zona pellucida	–	+++	+++	++	+++	–	–
Oocyte	+	++	+	+	++	–	–
Growing follicles							
Granulosa	++	–/+	–/+	++	–/+	–	–
Theca interna	++	–	–/+	++	++	–	–
Zona pellucida	++	+++	++	+++	+++	–	–
Oocyte	+	+++	+++	++	++	–	–
Granulosa in atretic follicles	+/++	+/+++	+++	++	++/+++	–	–
Interstitial glands	++	++[a]	++	++	++[a]	++/+++[a]	–
Mesothelium	++	–/+	++	+++	++	++	+
Endothelium of vessels	+	–/+	+++	++	++	–	–
Control group							
Growing follicles							
Granulosa cells	+/++	–/++	+	++/+++	+/++	–	–
Theca interna	+	–	+	++/+++	++/+++	–	–
Zona pellucida	–/+	+++	++	+++	+++	–	–
Oocyte	+	+++	++/+++	++	++	–	–
Granulosa in atretic follicles	+	–/+++	+	++	++/+++	–	–
Corpus luteum	+	++[a]	+++[a]	+++	+++[a]	++[a]	–
Interstitial glands	++	+[a]	+++	++	++[a]	++[a]	–
Mesothelium	++	–/+	+++	+++	+++	+++	++
Endothelium of vessels	+	–/+	++	++	+++	–	–

PO, polycystic ovaries.
(–) none, (+) weakly positive, (++) median positive and (+++) strongly positive.
[a]Strong in some cells.

lectin histochemistry pattern that coincides, in part, with those found in the bibliography (Salvetti et al. 2000; Parillo and Verini-Supplizi 2001; Parillo et al. 2003, 2005).

The ZP has been extensively studied in many species due to its importance in fertilization and specifically in the oocyte–sperm interaction. The ZP in growing follicles of both groups displays a moderate to strong positivity to WGA, RCA-I, PNA, Con-A and SBA lectins, indicating the occurrence of GlcNAc, Galβ1-4GlcNAc, sialic acid, α-D-Glc/α- D-Man and α/β-GalNAc. This result coincides with that described previously in rat (Avilés et al. 1994), rabbits and hares (Parillo and Verini-Supplizi 2001), human (Maymon et al. 1994), bovine (Velásquez et al. 2007), canine and feline (Parillo and Verini-Supplizi 1999) and equine ZP (Desantis et al. 2009). However, ZP of the oocytes from ovarian cysts shows a diminution in WGA, PNA and SBA reactivity and therefore an altered pattern of sialic acid, GlcNAc and α/β-GalNAc. Studies in various species (Avilés et al. 1994; Parillo et al. 1996; Verini-Supplizi et al. 1996) have concluded that the ZP is rich in sialic acid residues. Sialic acids, because they are negatively charged, are involved in the binding and transport of positively charged compounds, which play a role in the hydration of the ZP. These residues are also essential components of receptors: they mask specific glycoproteins that act as receptor sites for sperm recognition, keeping these glycoproteins inactive until the process of fertilization has taken place (Parillo and Verini-Supplizi 2001).

Granulosa cells of mammalian follicles synthesize GAGs at the time of antrum formation and secrete them into the follicular fluid (Salustri et al. 1999). These GAGs were identified as chondroitin sulphate, dermatan sulphate and a heparin-like substance (Salustri et al. 1999). Cumulus oophorus cells, which synthesize a wide spectrum of proteoglycans (Salustri et al. 1989), during the pre-ovulatory period, synthesize a large amount of hyaluronan, which is organized between the cells to form a muco-elastic matrix (Salustri et al. 1992).

Because GAGs have only a few sugar residues (Paulsson and Heinegard 1984; Mallinger et al. 1986), they can be freely accessed by lectin histochemistry (Pelli et al. 2010). The following terminal sugars are available for affinity labelling: *N*-acetyl-glucosamine in hyaluronic acid, *N*-acetyl-galactosamine for dermatan sulphate and chondroitin sulphate, beta-D-galactose-*N*-acetylgalactosamine for keratan sulphate. Based on the sugar affinity, the positivity of the WGA lectin suggests hyaluronic acid and/or keratan sulphate in the ovarian structures evaluated with variations between groups. On the other hand, the positivity to SBA and PNA suggest the presence of dermatan and/or chondroitin sulphate in growing, cystic and atretic follicles, corpus luteum and interstitial glands.

© 2013 Blackwell Verlag GmbH

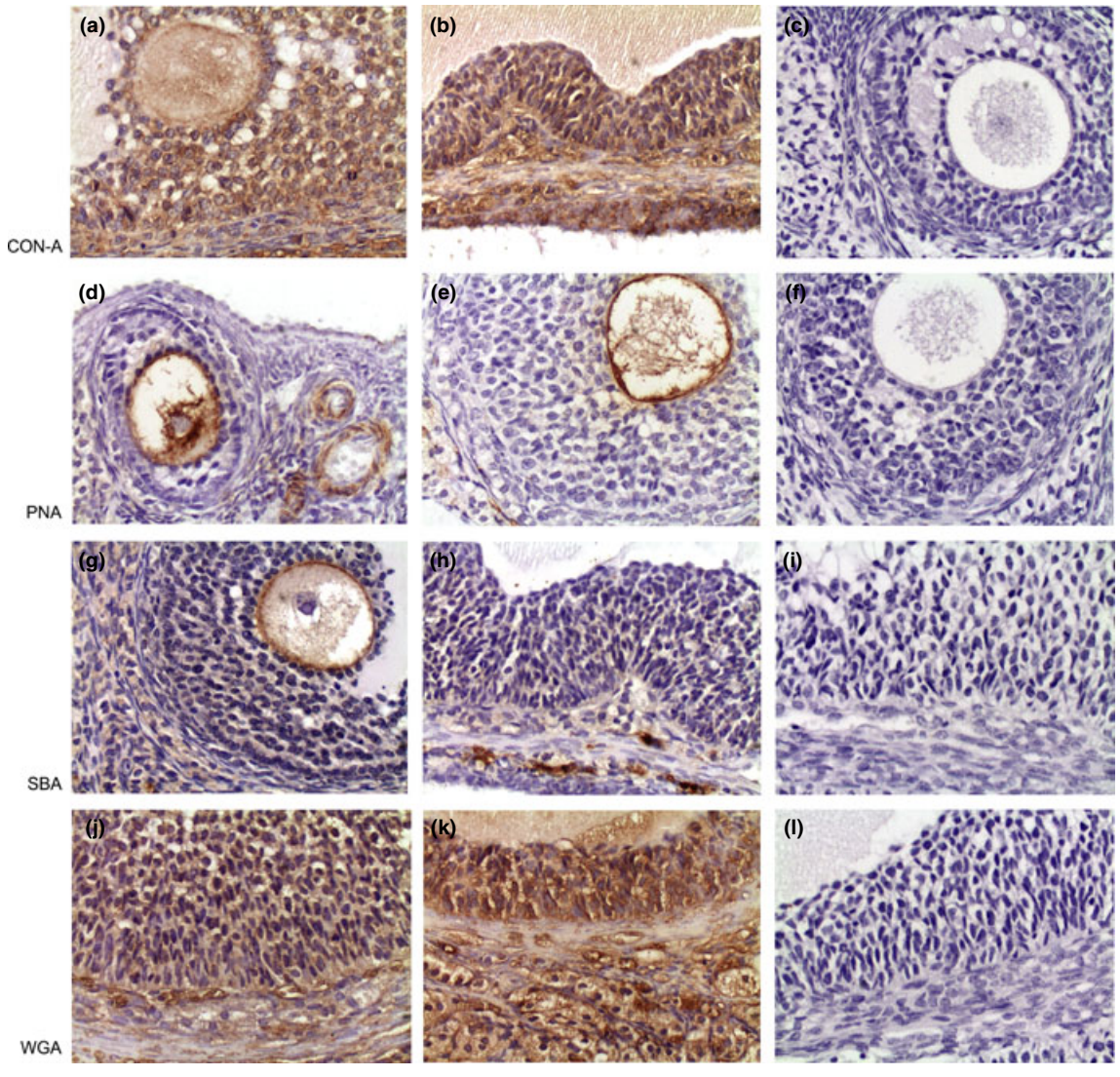

Fig. 1. It is observed moderate to strong staining with Con-A in the oocyte, granulosa cell layer and theca interna in a growing follicle from the control group (a) and in a follicular cyst from polycystic ovaries (PO) group (b). In (d) and (e), it is observed an intense staining with PNA in the oocyte and in the zona pellucida (ZP) with light staining in granulosa cells in growing follicles from the control group (d) and in the PO group (e). In g and h, it is observed weak staining with SBA in granulosa and theca interna cell layers in growing follicles from control group (g) and in follicular cysts (h) and an intense staining in the ZP (g). WGA showed a moderate to intense staining in the granulosa and theca interna of growing follicles from control group (j) and a strong staining in the same layers of the cysts (k). Negative controls of each lectin are shown (c, f, i, l)

Granulosa cells of growing follicles from PO group show a slight increment in the Con-A binding pattern and a diminution in RCA-I y WGA, while the granulosa of cyst shows an increment in CON-A, RCA-I and WGA with a slight decrease in the PNA-binding pattern with regard to control growing follicles. This result could indicate a gradual increase in α-D-Glc/α-D-Man residues. Stoica and O'Leary (1989) show similar results in normal structures of ovaries from rats with strong and uniform Con-A staining in normal antral follicles. Moreover, we have observed that atretic follicles of both groups behaved similarly except for an increase in labelling with SBA in the PO group. On the other hand, cysts show an increment in Con-A and WGA and a diminution in PNA and SBA with regard to atretic follicles in the same group. The theca interna layer presents a diminution in the staining with SBA, WGA and RCA-I in the growing follicles of PO group, while the cysts show an increment in SBA and WGA with regard to the control group. Con-A shows an increment in both growing and cystic follicles from PO group. Considering that extracellular matrix of the theca interna is composed largely of fibres but also by GAGs such as hyaluronate and sulphated GAGs, increased staining with these lectins could indicate increased production and accumulation of these matrix components.

If we take into account that ovulation is a complex process that is initiated by the LH surge and is controlled by the temporal and spatial expression of specific genes associated with formation of the cumulus matrix and the expression of proteases, changes in follicular wall component may affect this event. In this sense, hyaluronic acid is critical for cumulus oocyte matrix formation or cumulus cell differentiation, and their lack precludes expansion (Richards et al. 2002). Furthermore, ovulation is comparable to an inflammatory reaction and some molecules responsible for inducing the inflammatory cascade including proteins

© 2013 Blackwell Verlag GmbH

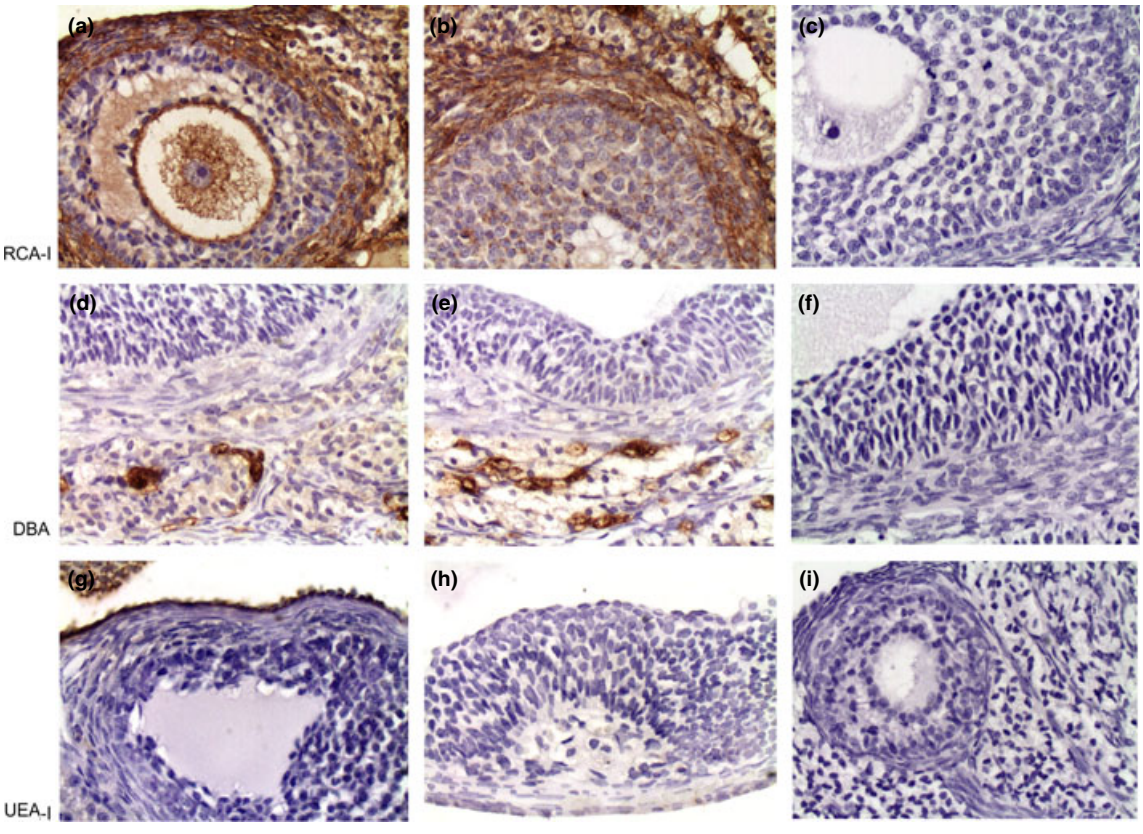

Fig. 2. It is observed moderate to strong staining with RCA-I in the oocyte, zona pellucida (ZP) and theca interna and moderate staining in the granulosa cells in a growing follicle from the control group (a) and in a follicular cyst from polycystic ovaries (PO) group (b). In (d) and (e), it is observed an intense staining with DBA in some cells in the interstitial glands with no staining in the follicular wall of growing follicles from the control group (d) and in the PO group (e). It was not observed staining with UEA-I in growing follicles from control group (g) and in follicular cysts (h). Negative controls of each lectin are shown (c, f, i)

with hyaluronidase enzymatic activity that catalyses the covalent linkage to extracellular matrix (Espey 1994; Jabbour et al. 2009). Therefore, changes in the composition of the matrix could affect normal oocyte maturation and ovulation, and follicular persistence triggering is the first step in the development of the cysts.

Also, in previous works, we have detected neutral carbohydrates in the intracellular, intercellular and interfibrous spaces, as in granulosa and theca interna layers, stained with PAS method. Particularly in granulosa cell layer, the intensity of staining was significantly lower in cystic ovaries. There were detected acidic GAGs in the follicular wall when Alcian blue staining was used (Salvetti et al. 2003). Granulosa was the more stained layer, and a significant reduction of the staining was found in the cystic ovaries. These data partially agree with the present work where a reduction in the binding of some lectins at the carbohydrates is evidenced in the granulosa of the ovarian cysts. However, there was an increase in the binding of certain lectins such as Con-A, WGA and RCA-I. In atretic follicles, it has been demonstrated that the concentrations of proteoglycans within the granulosa and the follicular fluid are higher than in the healthy follicle (Bellin and Ax 1984; Bellin et al. 1987; Huet et al. 1997; Salustri et al. 1999). Proteoglycan and hyaluronan syntheses by granulosa cells are significantly altered in response to gonadotrophins, which suggests that these macromolecules have defined functions during folliculogenesis. Follicle atresia is the result of an apoptotic process that occurs in granulosa cells, mainly for the lack of an appropriate gonadotrophin stimulus to generate the second messenger cAMP (Chun et al. 1994, 1996). High concentrations of GAGs in the culture medium inhibit gonadotrophin binding to rat granulosa cells and prevent the stimulation of adenylate cyclase (Salomon et al. 1978; Nimrod and Lindner 1980). Thus, upregulation of proteoglycan synthesis might indirectly participate to promote granulosa cell death by preventing gonadotrophin action (Bellin and Ax 1984). The response of granulosa cells to FSH in proteoglycan synthesis changes with follicular maturation. Granulosa cells in the large follicles have LH receptors, and LH seems to exert an inhibitory effect on proteoglycan synthesis by ovarian tissues (Gebauer et al. 1978). Such hormonal regulation may account for the observed decrease in total glycosaminoglycan concentration in the follicular fluid with the increase in the follicle size (Grimek et al. 1984; Salustri et al. 1999). Also, prostaglandins, epidermal growth factor and testosterone, like FSH, can stimulate proteoglycan synthesis by granulosa cells, and insulin-like growth factor-1 (IGF-1) increases the stimulatory effect of FSH (Adashi et al. 1986; Salustri et al. 1999). Thus, it is likely that these local factors participate in the regulation of proteoglycan synthesis *in vivo*. Whereas it is known that animals with PO have abnormalities in the

© 2013 Blackwell Verlag GmbH

hypothalamic-pituitary axis, it is likely that the changes found at the cellular level are closely related to hormone levels (Salvetti et al. 2004a,b).

In essence, the extracellular matrix defines or rather provides specialized microenvironments and, thus, determines both cell and tissue functions. It is not surprising that minor variations in extracellular matrix composition lead to alterations in the environment with consequent disturbances in ovarian function. Cystic ovaries show evident changes in the lectin-binding pattern, which probably are related to cystogenesis process and are different to the found in other ovarian alterations.

Acknowledgements

We are grateful to the staff of Biological Experimentation Center (FCV-UNL) for the animal care and help with generation of the experimental model; CGB, HHO, EJG and NRS are research career members of the Scientific Research Council (CONICET, Argentina). VM is fellow of the Argentine National Agency for the Promotion of Science and Technology (ANPCyT). This study was supported by grants from the National University of Litoral (CAI+D 2009. PI No 33-161 and 33-159).

Conflict of interest

None of the authors have any conflict of interest to declare.

Author contributions

CGB, HHO, NRS and EJG elaborated the experimental design. VM, HHO and NRS realized the experiments with animals. CGB and EJG processed the samples to lectinhistochemistry and observed the slides. CGB and NRS analyzed the results and written the manuscript. All the authors corrected the manuscript.

References

Abbott DH, Dumesic DA, Eisner JR, Colman RJ, Kemnitz JW, 1998: Insights into the development of polycystic ovary syndrome (PCOS) from studies in prenatally androgenised female rhesus monkeys. Trends Endocrinol Metab **9**, 62–67.

Adashi EY, Resnick CE, Svoboda ME, van Wyk JJ, Hascall VC, Yanagishita M, 1986: Independent and synergistic actions of somatomedin-C in the stimulation of proteoglycan synthesis by cultured granulosa cells. Endocrinology **118**, 456–458.

Avilés M, Martinez-Menarguez JA, Castells MT, Madrid JF, Ballesta J, 1994: Cytochemical characterization of oligosaccharide side chains of the glycoproteins of rat zona pellucida: an ultrastructural study. Anat Rec **239**, 137–149.

Baldissera SF, Motta LDC, Almeida MC, Antunes-Rodriguez J, 1991: Proposal of an experimental model for the study of polycystic ovaries. Braz J Med Biol Res **24**, 747–751.

Bell CM, Skerrow CJ, 1984: Factors affecting the binding of lectins to normal human skin. Br J Dermatol **111**, 517–526.

Bellin ME, Ax RL, 1984: Chondroitin sulfate: an indicator of atresia in bovine follicles. Endocrinology **114**, 428–434.

Bellin ME, Lenz RW, Steadman LE, Lenz RW, 1983: Proteoglycan production by bovine granulosa cells in vitro occurs in response to FSH. Mol Cell Endocrinol **29**, 51–65.

Bellin ME, Veldhuis JD, Ax RL, 1987: Follicular fluid glycosaminoglycans inhibit degradation of low-density lipoproteins and progesterone production by porcine granulosa cells. Biol Reprod **37**, 1179–1184.

Chapman JC, Wiebkin OW, Breed WG, 2000: Interspecific variation of zona pellucida glycoconjugates in several species of marsupial. J Reprod Fertil **119**, 111–120.

Chun SY, Billig H, Tilly JL, Furuta I, Tsafriri A, Hsueh AJ, 1994: Gonadotropin suppression of apoptosis in cultured preovulatory follicles: mediatory role of endogenous insulin-like growth factor I. Endocrinology **135**, 1845–1853.

Chun SY, Eisenhauer KM, Minami S, Billig H, Perlas E, Hsueh AJ, 1996: Hormonal regulation of apoptosis in early antral follicles: follicle-stimulating hormone as a major survival factor. Endocrinology **137**, 1447–1456.

Debray H, Decout D, Stecker G, Spik G, Montreuil J, 1981: Specificity of twelve lectins towards oligosaccharides and glycopeptides related to N-glycosylproteins. Eur J Biochem **117**, 41–55.

Desantis S, Ventriglia G, Zizza S, De Santis T, Di Summa A, De Metrio G, Dell'aquila ME, 2009: Lectin-binding sites in isolated equine cumulus-oocyte complexes: differential expression of glycosidic residues in complexes recovered with compact or expanded cumulus. Theriogenology **72**, 300–309.

Edwards DA, 1971: Neonatal administration of androstenedione, testosterone or testosterone propionate: effects on ovulation, sexual receptivity and aggressive behavior in female mice. Physiol Behav **6**, 223–228.

El-Mestrah M, Kan FWK, 2001: Distribution of lectin-binding glycosidic residues in the hamster follicular oocytes and their modifications in the zona pellucida after ovulation. Mol Reprod Dev **60**, 517–534.

Eriksen GV, Carlstedt I, Morgelin M, Uldbjerg N, Malmstrom A, 1999: Isolation and characterization of proteoglycans from human follicular fluid. Biochem J **340**, 613–620.

Espey LL, 1994: Current status of the hypothesis that mammalian ovulation is comparable to an inflammatory reaction. Biol Reprod **50**, 233–238.

Gebauer H, Lindner HR, Amsterdam A, 1978: Synthesis of heparin-like glycosaminoglycans in rat ovarian slices. Biol Reprod **18**, 350–358.

Grimek HJ, Bellin ME, Ax RL, 1984: Characteristics of proteoglycans isolated from small and large bovine ovarian follicles. Biol Reprod **30**, 397–409.

Grootenhuis AJ, Philipsen HLA, de Breet-Grijsbach JTM, van Duin M, 1996: Immunocytochemical localization of ZP3 in primordial follicles of rabbit, marmoset, rhesus monkey and human ovaries using antibodies against human ZP3. J Reprod Fertil **50**, 43–54.

Hirabayashi J, 2008: Concept, strategy and realization of lectin-based glycan profiling. J Biochem **144**, 139–147.

Huet C, Monget P, Pisselet C, Monniaux D, 1997: Changes in extracellular matrix components and steroidogenic enzymes during growth and atresia of antral ovarian follicles in the sheep. Biol Reprod **56**, 1025–1034.

Jabbour HN, Sales KJ, Catalano RD, Norman JE, 2009: Inflammatory pathways in female reproductive health and disease. Reproduction **138**, 903–919.

Jones HM, Vernon MW, Rush ME, 1987: Systematic studies invalidate the neonatally androgenized rat as a model for polycystic ovary disease. Biol Reprod **36**, 1253–1265.

Kalra SP, Kalra PS, 1983: Neural regulation of luteinizing hormone secretion in the rat. Endocr Rev **4**, 311–351.

Mahajan DK, 1988: Polycystic ovarian disease: animals models. Endocrinol Metab Clin North Am **17**, 705–732.

Mahesh VB, Mills TM, Bagnell CA, Conway BA, 1987: Animal models for study of polycystic ovaries and ovarian atresia. In: Mahesh VB, Dhindsa DS, Anderson E, Kalra SP (eds), Regulation of Ovarian and Testicular Function. Plenum, New York, pp. 237–258.

Mallinger R, Geleff S, Böck P, 1986: Histochemistry in cartilage ground substance. Histochem Cell Biol **85**, 121–127.

Maymon BB, Maymon R, Ben-Nun I, Ghetler Y, Shalgi R, Skutelsky E, 1994: Distribution of carbohydrates in the zona pellucida of human oocytes. J Reprod Fertil **102**, 81–86.

McArthur ME, Irving-Rodgers HF, Byers S, Rodgers RJ, 2000: Identification and immunolocalization of decorin, versican, perlecan, nidogen, and chondroitin sulfate proteoglycans in bovine small-antral ovarian follicles. Biol Reprod **63**, 913–924.

Mueller PL, Schreiber JR, Lucky AW, Schulman JD, Rodbard D, Ross GT, 1978: Follicle-stimulating hormone stimulates ovarian synthesis of proteoglycans in the estrogen stimulated hypophysectomized immature female rat. Endocrinology **102**, 824–831.

© 2013 Blackwell Verlag GmbH

Nimrod A, Lindner H, 1980: Heparin facilitates the induction of LH receptors by FSH in granulosa cells cultured in serum-enriched medium. FEBS Lett **119**, 155–157.

Ortega HH, Amable P, Salvetti NR, Dallard BE, Baravalle C, Barbeito CG, Gimeno EJ, 2007: Intraovarian localization of growth factors in induced cystic ovaries in rats. Anat Histol Embryol **36**, 94–102.

Parillo F, Verini-Supplizi A, 1999: Glycohistochemical investigation of canine and feline zonae pellucidae of preantral and antral oocytes. Acta Histochem **101**, 127–146.

Parillo F, Verini-Supplizi A, 2001: Glycohistochemistry of the zona pellucida of developing oocytes in the rabbit and hare. Res Vet Sci **70**, 257–264.

Parillo F, Stradaioli G, Dall'aglio C, Verini Supplizi A, 1996: Characterization of the complex carbohydrates in the zona pellucida of mammalian oocytes using lectin histochemistry. Vet Res Commun **20**, 225–236.

Parillo F, Dall'Aglio C, Verini Supplizi A, Ceccarelli P, Gargiulo AM, 2003: Immunogold study on lectin binding in the porcine zona pellucida and granulosa cells. Eur J Histochem **47**, 353–358.

Parillo F, Zelli R, Verini Supplizi A, Fagioli O, Gargiulo AM, 2005: Topographical localisation of glucidic residues and their variations in the canine zona pellucida during folliculogenesis. J Mol Histol **36**, 131–137.

Pauli JM, Raja-Khan N, Wu X, Legro RS, 2011: Current perspectives of insulin resistance and polycystic ovary syndrome. Diabet Med **28**, 1445–1454.

Paulsson M, Heinegard D, 1984: Noncollagenous cartilage proteins current status of an emerging research field. Coll Relat Res **3**, 219–229.

Pelli AA, Cinelli LP, Mourão PA, de Brito-Gitirana L, 2010: Glycosaminoglycans and glycoconjugates in the adult anuran integument (*Lithobates catesbeianus*). Micron **41**, 660–665.

Rath D, Töpfer-Petersen E, Michelmann HW, Schwartz P, Ebeling S, 2005: Zona pellucida characteristics and sperm-binding patterns of 'in vivo' and 'in vitro' produced porcine oocytes inseminated with differently prepared spermatozoa. Theriogenology **63**, 352–362.

Regassa F, Mengesha D, Dargie M, Tolosa T, 2009: Abattoir evidence on association between uterine and ovarian abnormalities in Ethiopian highland ewes. Anim Reprod Sci **111**, 384–390.

Richards JS, Russell DL, Ochsner S, Espey LL, 2002: Ovulation: new dimensions and new regulators of the inflammatory-like response. Annu Rev Physiol **64**, 69–92.

Rodgers RJ, Irving-Rodgers HF, Russell DL, 2003: Extracellular matrix of the developing ovarian follicle. Reproduction **126**, 415–424.

Salomon Y, Amir Y, Azulai R, Amsterdam A, 1978: Modulation of adenylate cyclase activity by sulfated glycosaminoglycans. I. Inhibition by heparin of gonadotropin-stimulated ovarian adenylate cyclase. Biochim Biophys Acta **554**, 262–272.

Salustri A, Yanagishita M, Hascall VC, 1989: Synthesis and accumulation of hyaluronic acid and proteoglycans in the mouse cumulus cell-oocyte complex during follicle stimulating hormone-induced mucification. J Biol Chem **264**, 13840–13847.

Salustri A, Yanagishita M, Underhill C, Laurent TC, Hascall VC, 1992: Localization and synthesis of hyaluronic acid in the cumulus cells and mural granulosa cells of the preovulatory follicles. Dev Biol **151**, 541–551.

Salustri A, Camaioni A, Di Giacomo M, Fulop C, Hascall VC, 1999: Hyaluronan and proteoglycans in ovarian follicles. Hum Reprod Update **5**, 293–301.

Salvetti NR, Ricci N, Dallard BE, Lorente JA, Iguzquiza I, Ortega HH, 2000: Lectinhistochemical and cytometrical evaluation of the corpus luteum of the rat at the end of pregnancy. Anat Histol Embryol **29**, 129–134.

Salvetti NR, Canal AM, Gimeno EJ, Ortega HH, 2003: Histochemical study of the extracellular matrix components in the follicular wall of induced polycystic ovaries. Braz J Morphol Sci **20**, 93–100.

Salvetti NR, Canal AM, Gimeno EJ, Ortega HH, 2004a: Polycystic Ovarian Syndrome: temporal characterization of the induction and reversion process in an experimental model. Braz J Vet Res Anim Sci **41**, 389–395.

Salvetti NR, Gimeno EJ, Lorente JA, Ortega HH, 2004b: Expression of cytoskeletal proteins in the follicular wall of induced ovarian cysts. Cells Tissues Organs **178**, 117–125.

Salvetti NR, Baravalle C, Mira GA, Gimeno EJ, Dallard BE, Rey F, Ortega HH, 2009a: Heat shock protein 70 and sex steroid receptors in the follicular structures of induced ovarian cysts. Reprod Domest Anim **44**, 805–814.

Salvetti NR, Panzani CG, Gimeno EJ, Neme LG, Alfaro NS, Ortega HH, 2009b: An imbalance between apoptosis and proliferation contributes to follicular persistence in polycystic ovaries in rats. Reprod Biol Endocrinol **7**, 68.

Salvetti NR, Stangaferro ML, Palomar MM, Alfaro NS, Rey F, Gimeno EJ, Ortega HH, 2010: Cell proliferation and survival mechanisms underlying the abnormal persistence of follicular cysts in bovines with cystic ovarian disease induced by ACTH. Anim Reprod Sci **122**, 98–110.

Silvia WJ, Hatler TB, Nugent AM, Laranja DA, Fonseca LF, 2002: Ovarian follicular cysts in dairy cows: an abnormality in folliculogenesis. Domest Anim Endocrinol **23**, 167–177.

Silvia WJ, McGinnis AS, Hatler TB, 2005: A comparison of adrenal gland function in lactating dairy cows with or without ovarian follicular cysts. Reprod Biol **5**, 19–29.

Singh KB, 1969: Induction of polycystic ovarian disease in rats by continuous light. Am J Obstet Gynecol **104**, 1079–1083.

Singh KB, 2005: Persistent estrus rat models of polycystic ovary disease: an update. Fertil Steril **84**(Suppl. 2), 1228–1234.

Stoica G, O'Leary M, 1989: Lectin binding sites in normal rat ovary and ENU-induced Sertoli cell tumors of the ovaries. Anticancer Res **9**, 679–685.

Sun YL, Zhang J, Ping ZG, Wang CQ, Sun YF, Chen L, Li XY, Li CJ, Zhu XL, Liu Z, Zhang W, Zhou X, 2011: Relationship between apoptosis and proliferation in granulosa and theca cells of cystic follicles in sows. Reprod Domest Anim **47**, 601–608.

Szukiewicz D, Uilenbroek JT, 1998: Polycystic ovary syndrome- searching for an animal model. J Med **29**, 259–275.

Toda N, Doi A, Jimbo A, Matsumoto I, Seno N, 1981: Interaction of sulphated glycosaminoglycans with lectins. J Biol Chem **256**, 5345–5349.

Vanholder T, Opsomer G, De Kruif A, 2006: Aetiology and pathogenesis of cystic ovarian follicles in dairy cattle: a review. Reprod Nutr Dev **46**, 105–119.

Velásquez JG, Canovas S, Barajas P, Marcos J, Jiménez-Movilla M, Gutiérrez Gallego R, Ballesta J, Avilés M, Coy P, 2007: Role of sialic acid in bovine sperm-zona pellucida binding. Mol Reprod Dev **74**, 617–628.

Verini-Supplizi A, Monaci M, Stradaioli G, Greve T, Parillo F, 1996: Identification of glycoconjugates in the zona pellucida of in vitro matured and tubal unfertilized bovine oocytes by lectin histochemistry. Anim Reprod Sci **43**, 99–111.

Walters KA, Allan CM, Handelsman DJ, 2012: Rodent models for human polycystic ovary syndrome. Biol Reprod **86**, 1–12.

Yanagishita M, Rodbard D, Hascall VC, 1979: Isolation and characterization of proteoglycans from porcine ovarian follicular fluid. J Biol Chem **254**, 911–920.

Yonezawa N, Mitsui S, Kudo K, Nakano M, 1997: Identification of an N-glycosylated region of pig zona pellucida glycoprotein ZPB that is involved in sperm binding. Eur J Biochem **248**, 86–92.

Zurn AD, 1982: Identification of glycolipid binding sites for soybean agglutinin and differences in the surface glycolipids of cultured adrenergic and cholinergic sympathetic neurons. Dev Biol **94**, 483–498.

Submitted: 13 Aug 2012; Accepted: 10 Mar 2013

Author's address (for correspondence): NR Salvetti, Facultad de Ciencias Veterinarias, Universidad Nacional del Litoral, RP Kreder 2805, CP 3080 Esperanza, Santa Fe, Argentina.
E-mail: salvetti@fcv.unl.edu.ar

© 2013 Blackwell Verlag GmbH