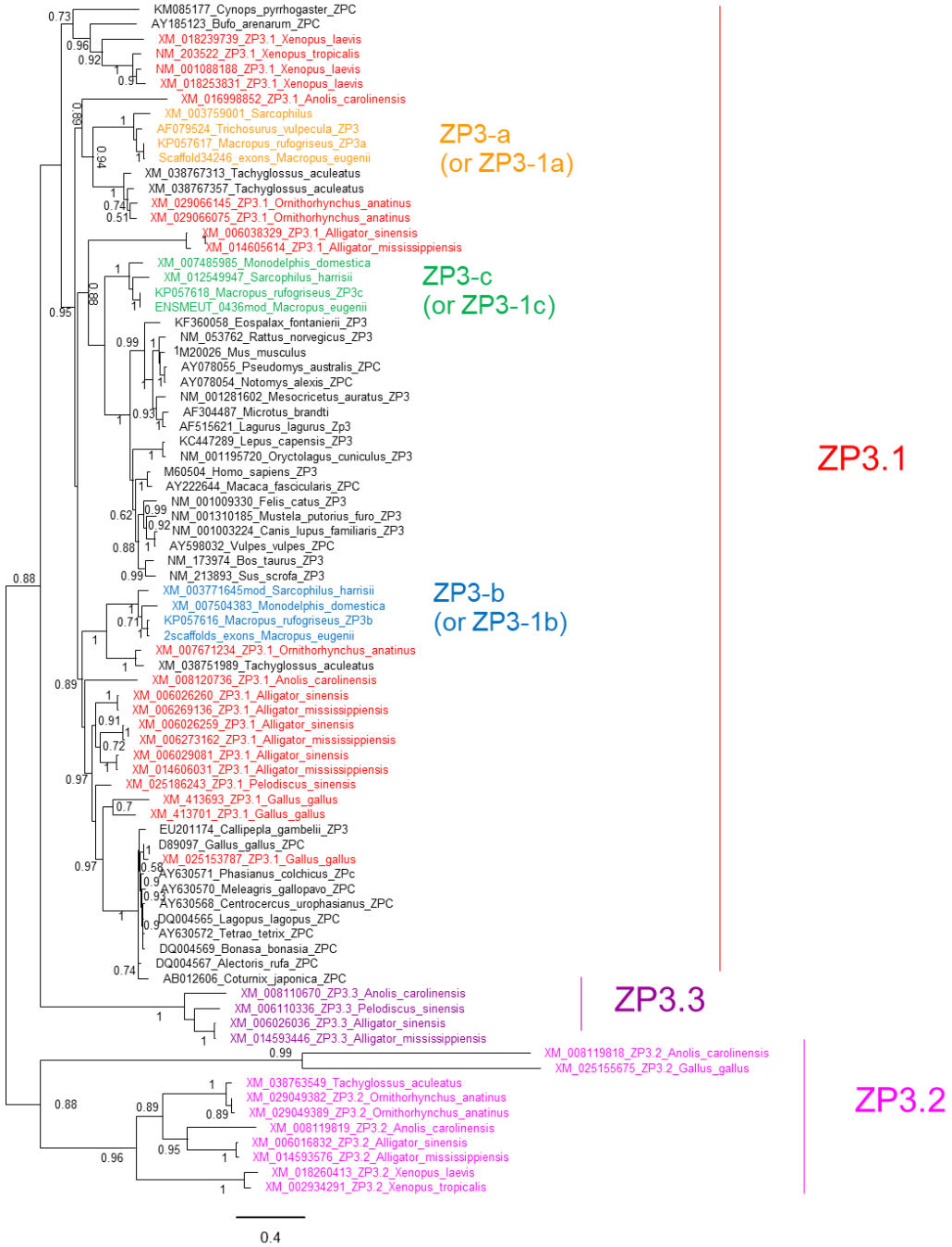

Figure S1. Phylogeny of ZP3 genes in tetrapodes reconstructed with PhyML. Sequences in red, pink and violet correspond respectively to ZP3.1, ZP2.2 and ZP3.3 of Feng et al., 2018. Sequences in orange, blue and green correspond respectively to the marsupial ZP3-a, ZP3-b and ZP3-c of Moros-Nicolas et al., 2018. The supports (aLRT values) are indicated on the nodes for values above 0.5.

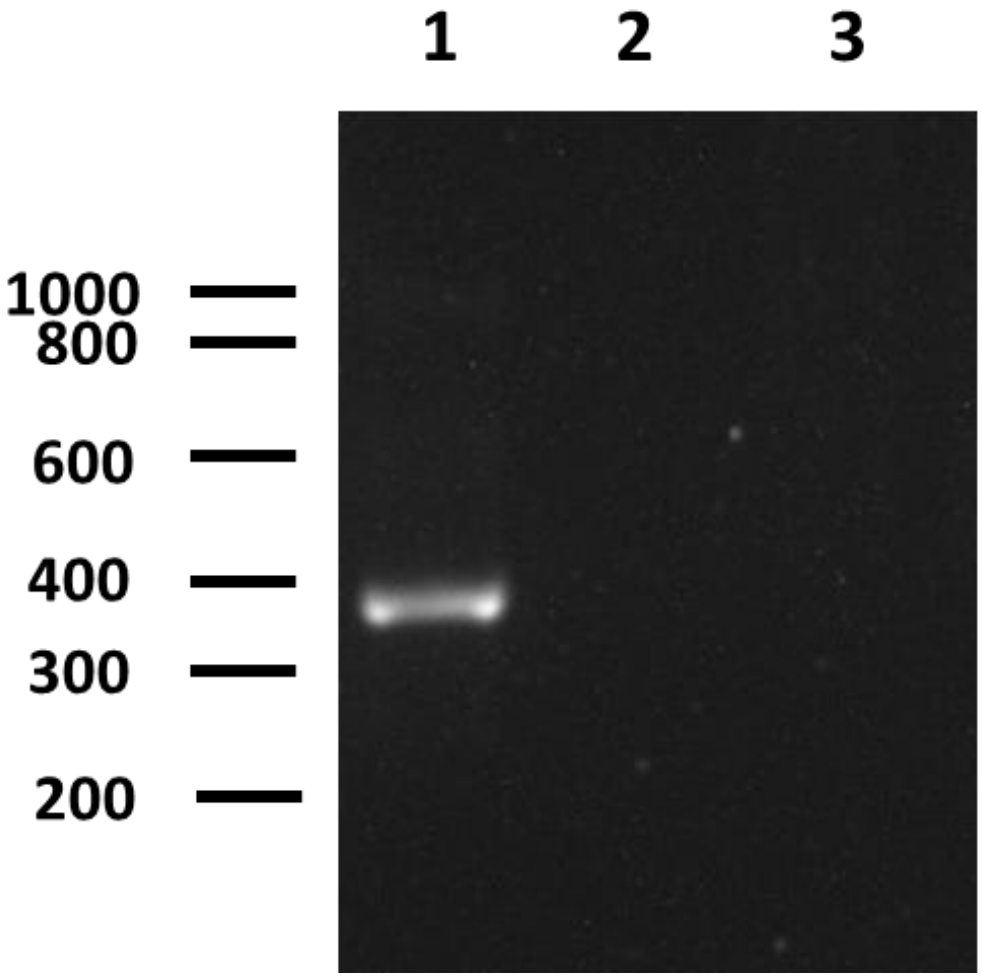

Figure S2. Analysis of *ZPAX* gene expression in Bennett's wallaby ovary as determined by reverse transcription–polymerase chain reaction (RT-PCR). In line 1 a fragment of *GAPDH* gene is shown as control. In lines 2 and 3 no fragments of *ZPAX* gene were amplified.

Table S1. Lectin histochemistry of the ZP from different mammalian species before and after different treatments.

LECTIN	Human	Mouse	Rat	Hamster	Rabbit	Hare	Cat	Dog	Pig	Cow
Con A	+ # [1] + # int. fail. [2] + # [3]	+ # unif. [4] ± ext. [5] + # [6]	+ # unif. [4] + # unif. [11] + unif. [12] ± ext. [5]	+ # unif. [4] ± ext. [5]	± ext. [5] - [16]	- [16]	- [5] - [17]	- [5] - [17] - primary and secondary [18] ± int. [19] - primary [19]	- [5] - [20] + unif. antral [21] - primordial, primary, multilaminar and antral [22] - *D [22] - preovulatory [23] + IVM [23]	- [26] - IVM [26] + # ext. and int. [26] - [27]
LCA	+ # [1]	± [5]	- # [11] ± [5]	± [5]	+ ext. [5] - [16]	- [16]	- [5] - [17]	± [5] - [17]	± [5]	
WGA	+ # int. and ext. [1] + # fail. [2] + # [3] ± #*S [3]	+ # ext. [4] + ext. and int. [5] + # unif. [7] + #*S int. ± #*S ext. [7] + E unif. [7] + E*S int. ± E*S ext. [7] + throughout follicular development [8]	+ # ext. [4] - [11] ± # int. and ext. [11] + int. and ext. [12] ± unif. *S [12] + [5] + # int. + # ext. [13] + #*S int. ± #*S ext. [13] + E int. + E ext. [13] + E*S int. ± E*S ext. [13]	+ # ext. [4] + ext. and int. [5] ± early primary + int. ± ext. unilaminar matured to multilaminar [14] + *S unif. [14] + # unif. [14] + #*S unif. [14] + # unif. [15] + #*S unif. [15] + E unif. [15] + E*S unif. [15]	+ [5] + ext. and int. [16] + unif. preantral [16] + *S ext. and int. [16] + *KOH/S ext. and int. [16] + *D ext. and int. [16]	+ unif. [16] + *S unif. [16] + *KOH/S unif. [16] + *D unif. [16]	- [5] + ext. and int. [17] + unif. preantral [17] + *S ext. and int. [17] + *S unif. preantral [17]	+ [5] + ext. and int. [17] + unif. preantral [17] + *S ext. and int. [17] + *S unif. preantral [17] - primary and secondary [18] + ext. [19] + *S ext. [19] + *KOH/S ext. [19]	+ [5] + ext. and int. [20] + unif. antral [21] + *S unif. antral [21] + primordial, primary, multilaminar and antral [22] + *D [22] + *KOH/S preovulatory [23] + IVM*KOH/S [23] + [24]	+ ext. and int. [26] + IVM ext. and int. [26] + E ext. and int. [26] + [27]
S-WGA	+ # med. [1]	+ ext. [5]	+ ext. [11] + # unif. [11] + ext.	+ ext. [5]	+ ext. [5]		- [5] + ext. and int. [17]	- [5] - primary [18]	- [5]	

			[5]					+ secondary [18]		
PNA	- # [1] + #*S [1] - # fail. [2] - # [3] + #*S [3]	+ [5] + *S [5] + # int. ± # ext. [7] + E int. ± E ext. [7] + # [6]	+ ext. [11] + # unif. [11] + int. ± ext. [12] + unif. *S [12] + [5] + *S [5] + # int. ± # ext. [13] + E int. ± E ext. [13]	+ ext. [5] + *S ext. [5] ± early primary + int. ± ext. unilaminar matured to multilaminar [14] ± # unif. [14] ± # unif. [15] ± E unif. [15]	± [5] - *S [5] + ext. [16] + *S unif. preantral [16] + *D ext. [16] + *D unif. preantral [16] + *D/S unif. [16]	- [16] + *D unif. [16] + *D/S unif. [16]	- [5] - *S [5] - [17] - *S [17] - *S unif. preantral [17] + *D unif. [17] + *D unif. preantral [17] + *D/S unif. [17] + *D/S unif. preantral [17]	- [5] - *S [5] - [17] - *S [17] + *D/S unif. [17] - [19]	- [5] - *S [5] - [20] - *S [20] + *KOH/S unif. [20] - *D antral [25] + *D/S unif. antral [25] - antral [21] ±*S unif. antral [21] ± antral [22] - primordial, primary and multilaminar [22] + *D [22] - preovulatory [23] - IVM [23] - [24]	- [26] - *S [26] + *KOH/S unif. [26] - IVM [26] - IVM*S [26] + IVM*KOH/S unif. [26] - E [26] + E*S ext. and int. [26] + E*KOH/S ext. and int. [26] ± *D ext. antral [25] + *D/S unif. antral [25] - antral [28]
RCA-I	+ # [1] + # [3]	+ # ext. [4] + ext. [5] + # int. ± # ext. [7] + E int. ± E ext. [7] + throughout follicular development [8]	+ # ext. [4] - [11] + # int. [11] ± int. + ext. [12] + unif. *S [12] + ext. [5] + # int. ± # ext. [13] + E int. ± E ext.	+ # ext. [4] + ext [5] ± early primary + int. ± ext. unilaminar matured to multilaminar [14] + # int. ± # ext. [14] + # int. ± # ext. [15] + E int. ± E ext. [15]	+ ext. [5] + [16] + *D/S unif. [16]	- [16]	+ ext. [5] - [17] + *S int. [17] + *S unif. preantral [17] + *KOH/S unif. [17] + *D unif. [17]	- [5] - [17] + *S ext. and int. [17] + *S unif. preantral [17] ± unif. [19] + primary [19] + *S int. [19] + *D ext. and int. [17]	+ ext. [5] + int. [20] + *S unif. [20] + *KOH/S unif. [20] - *D antral [25] + *D/S unif. antral [25] + int. antral [21] +*S int. and ext. antral	- [26] - *S [26] + *KOH/S unif. [26] - IVM [26] - IVM*S [26] + IVM*KOH/S unif. [26] - E [26] - E*S [26]

			[13]					+ *D unif. preantral [17] + *D/S ext. and int. [17] + *D/S unif. preantral [17]	[21] + primordial, primary, multilaminar and antral [22] + *D [22] + preovulatory [23] + IVM [23] + [24]	+ E*KOH/S unif. [26] + *D ext. antral [25] + *D/S unif. antral [25]
GS-I	- # [1] - # fail. [2]	+ int. [5]	+ # unif. [11] + [5]	- [5]	- [5]		- [5]	- [5]	- [5]	+ [27]
GS-II	- # fail. [2]									
SBA	- # [1] - # fail. [2] - # [3] + #*S [3]	+ [5] + unif. throughout follicular development [9] + # [9]	- [11] + # unif. [11] ± int. + ext. [12] + unif. *S [12] + [5]	+ [5]	- [5] + ext. [16] + *S ext. [16] + *S unif. preantral [16] + *KOH/S unif. [16] + *D ext. and int. [16] + *D unif. preantral [16] + *D/S unif. [16]	- [16] + *S unif. [16] + *D unif. [16] + *D/S unif. [16]	- [5] - [17] + *S unif. [17] + *D ext and int. [17] + *D unif. preantral [17] + *D/S unif. [17]	- [5] - [17] + *S ext. and int. [17] + *S unif. preantral [17] - [19] + *S ext. and int. [19] + *KOH/S ext. and int. [19] + *D ext. and int. [17] + *D unif. preantral [17] + *D/S ext. and int. [17] + *D/S unif. preantral [17]	- [5] - [20] + *S unif. [20] + *KOH/S unif. [20] - *D ext. antral [25] + *D/S unif. antral [25] + int. and med. antral [21] - multilaminar [22] ± antral [22] + primordial and primary [22] + *D [22] + preovulatory [23] + IVM [23] - [24]	- [26] + *S ext. and int. [26] + *KOH/S unif. [26] - IVM [26] + IVM*S ext. and int. [26] + IVM*KOH/S unif. [26] + E ext. [26] + E*S ext. and int. [26] + E*KOH/S unif. [26] + *D ext. antral [25] + *D/S unif. antral [25] ± antral [28] + *S antral [28]
DBA	- #	+ int.	-	-	-	-	-	-	-	-

	[1] - # fail. [2] - # [3] - #*S [3]	[5] + unif. throughout follicular development [9] + # [9]	[11] + # unif. [11] - [5] - # [13] - E [13]	[5] - [14] ± # unif. [14] ± # unif. [15] ± E unif. [15]	[5] - [16]	[16]	[5] - [17]	[5] - [17] - [19]	[5] - [20] - *S [20] - *KOH/S [20] - *D/S antral [25] - int. antral [21] - primordial, primary, multilaminar and antral [22] - *D [22] - preovulatory [23] - IVM [23] - [24]	[26] - *S [26] - *KOH/S [26] - IVM [26] - IVM*S [26] - IVM*KOH/S [26] - E [26] - E*S [26] + E*KOH/S [26] - *D/S antral [25] - antral [28]
UEA-I	- # [1] - # fail. [2] - # [3] - #*S [3]	- [5] - [8] - [10] - # [10]	- [11] ± # [11] - [5] - # [13] - E [13] - [10] - # [10]	- [5] - [10] - # [10] - [14] - # [14] - # [15]	- [5] - [16]	- [16]	- [5] - [17]	- [5] - [17] ± int. [19] - primary [19] - primary and secondary [18]	- [5] - [20] ± int. antral [21] -primordial and primary [22] + multilaminar and antral [22] + *D [22] - preovulatory [23] - IVM [23] +/- [24]	- [26] - IVM [26] - E [26] - [27]
AAA	+ # [3]	+ # int. ± # ext. [7] + E int. ± E ext. [7] + throughout follicular development [8] +	+ unif. [12] + # int. ± # ext. [13] ± E unif. [13] + [10] + *α-gal.	+ [10] + *α-gal. [10] + # [10] + *α-gal. # [10]					- primordial, primary, multilaminar and antral [22] + *D [22]	

		[10] + *α-gal. [10] + # [10] + *α-gal. # [10]	[10] + # [10] + *α-gal. # [10]	+ throughout follicular development [14] + # [14] + # unif. [15] + E int. ± E ext. [15]					
DSA	+ # [3]	+ # ext. ± # int. [7] + E int. ± E ext. [7] + throughout follicular development [8]	+ unif. [12] + # int. ± # ext. [13] + E int. ± E ext. [13]	± early primary + int. ± ext. unilaminar matured to multilaminar [14] + # int. ± # ext. [14] + # int. ± # ext. [15] + E int. ± E ext. [15]					
LFA	+ # [3] - #*S [3]	+ # int. ± # ext. [7] + E int. ± E ext. [7] + throughout follicular development [8] - *S [8]	+ unif. [12] + # int. ± # ext. [13] + E int. ± E ext. [13]					- primordial and primary [22] + multilaminar and antral [22] - *D primordial and primary [22] + *D multilaminar and antral [22]	+ antral unif. [28] - *S antral [28]
MAA	+ # [3] - #*S [3]	+ # int. ± # ext. [7] + E int. ± E ext. [7] + throughout follicular development [8] - *S [8]	+ unif. [12] + # int. ± # ext. [13] + E int. ± E ext. [13]	± early primary + int. ± ext. unilaminar matured to multilaminar [14] + # unif. [14] - #*S [14] + # unif. [15] - #*S [15] + E unif. [15]				-primordial [22] ± primary [22] + multilaminar and antral [22] - *D primordial [22] ± *D primary [22] + *D multilaminar and antral [22]	+ antral unif. [28] - *S antral [28]
PHA-E	+ # [3]								
PHA-L	+ #	+ #							

	[3]	[6]								
BSA-I-B4	- # [3]	± # int. - # ext. [7] ± E int. - E ext. [7]		- [14] ± # unif. [14] + unif. [15] + E int. ± E ext. [15]						
GNA	- # [3]	- [8]	- # [13] - E [13]	- [14] - # [14] - # [15]					- primordial, primary, multilaminar and antral [22] - *D primordial, primary, multilaminar and antral [22]	
HPA	- # [3] - #*S [3]	+ # int. - # ext. [7] + E int. - E ext. [7] + int. primary to preovulatory [8] + *S throughout follicular development [8]	- # [13] - E [13]	- [14] ± *S [14] + # [14] + # unif. [15] + E int. ± E ext. [15]					- primordial, primary, multilaminar and antral [22] - *D [22] - preovulatory [23] - IVM [23]	- antral [28]
LTA	- # [3] + #*S [3]	- [8] - [10] - # [10]	- # [13] - E [13] - [10] - # [10]	- [10] - # [10] - [14] - # [14] - # [15]	- [16]	- [16]	- [17]	- [17] - [19]	- [20] - int. antral [21] - primordial, primary, multilaminar and antral [22] - *D [22] - preovulatory [23] - IVM [23]	- [26] - IVM [26] - E [26]
MAL II									+ preovulatory [23] - *KOH/S preovulatory [23] + IVM [23] - *KOH/S IVM [23]	

SNA	- # [3]	- [8]	- # [13] - E [13]	- [14] - # [14] - # [15]					- primordial, primary, multilaminar and antral [22] - *D primordial and primary [22] + *D multilaminar and antral [22] - preovulatory [23] - *KOH/S preovulatory [23] + IVM ext. and int. [23] - *KOH/S IVM [23]	+ antral unif. [28] - *S antral [28]
STA	- # [3]									
GSA-II					- [16]	- [16]	- [17]	- [17]	- [20] - *D antral [25] ± *D/S unif. antral [25] - preovulatory [23] - IVM [23]	- [26] - IVM [26] - E [26] - *D antral [25] + *D/S unif. antral [25]
GSA-IB4					- [16]	- [16]	- [17] + *D unif. [17] + *D/S unif. [17]	- [17] + *D/S unif. [17]	± *D ext. antral [25] + *D/S unif. antral [25]	+ *D ext. antral [25] + *D/S unif. antral [25]
BPA	- # fail. [2]									
MPA	+ # fail. [2] + # [3]									
PSA	+ # int. fail. [2]							- primary [18] + secondary [18]		
Jacalin	+ # [3]									
ECL								- primary [18] + secondary [18]		
GSL-I								- primary and secondary [18]		

GSL-II								± primary [18] + secondary [18]		
LPA										+ [27]
LEL								± primary [18] + secondary [18]		
DSL								- primary and secondary [18]		
SJA								- primary and secondary [18]		
PVL								- primary and secondary [18]		
PVE								- primary and secondary [18]		
VVA								± primary and secondary [18]		

Positive binding: **+**; Weakly positive binding: **±** (sometimes used for showing intensity differences between the external and internal ZP surface**)**; Negative binding: -

Binding localized in the external ZP surface: **ext**.; internal ZP surface: **int.**; medium ZP: **med.** or uniform binding: **unif.**

Most analysis were carried out using sections of ovaries. Some of them used isolated tubal unfertilized oocytes: **#;** fertilized eggs: **E** or *in vitro* matured oocytes: **IVM.** Oocytes failing to fertilize in vitro: **fail.**

*****: lectin binding procedures were preceded by different pre-treatments. Sialidase digestion: **S**; Saponification/sialidase digestion: **KOH/S**; Desulphation process: **D;** Desulphation/sialidase digestion: **D/S;** α-galactosidase: **α-gal.**

Some references distinguish between **primordial**, **primary**, **secondary**, **multilaminar** and **antral** follicles. Some lectins bound the ZP of ovarian oocytes starting from the early stages of follicular growth (primary follicles) to the Graafian follicle stage. That is designated as: **Throughout follicular development.**

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Table S2. Lectin histochemistry of the ZP from different mammalian and marsupial species before and after different treatments.

LECTIN	Wild boar	Fallow deer	Red deer	Roe deer	Buffalo	Fat-tailed dunnart	Southern brown bandicoot	Grey short-tailed opossum	Brushtail possum	Ringtail possum	Koala	Eastern grey kangaroo
Con A	- [1] - *S [1] - *KOH/S [1] - *D [1]	- [2] - *S [2] - *KOH/S [2] - *D [2]	- [2] - *S [2] - *KOH/S [2] - *D [2]	- [2] - *S [2] - *KOH/S [2] - *D [2]	- [3] - *S [3]	- [4]	- [4]	- [4]	- [4]	- [4]	- [4]	- [4]
LCA	- [1] - * S [1] - *KOH/S [1] - *D [1]				- [3] - * S [3] - *KOH/S [3] - *D [3]							
WGA	+ ext. and int. [1] + *S ext. and int. [1] + *KOH/S ext and int. [1]	+ ext. and int. [2] + *S ext. and int. [2] + *KOH/S ext and int. [2]	+ ext. and int. [2] + unif. preantral [2] + *S ext. and int. [2] + *KOH/S ext. and int. [2]	+ ext. and int. [2] + unif. preantral [2] + *S ext. and int. [2] + *KOH/S ext. and int. [2]	+ ext. and int. [3] + *S [3] + *KOH/S [3]	+ [4] + *S [4]	+ [4] + *S [4]	+ [4] + *S [4]	+ [4] + *S [4]	+ [4] + *S [4]	+ [4] + *S [4]	+ [4] + *S [4]
PNA	- [1] + *KOH/S [1] + *D ext. [1] + *D unif. preantral [1] + *D/S [1]	- [2] + *S ext and int. [2] + *S unif. preantral [2] + *KOH/S unif. [2] + *D ext. [2] + *D unif. preantral [2]	- [2] + *S ext. and int. [2] + *S unif. preantral [2] + *KOH/S ext. and int. [2] + *KOH/S unif. preantral [2] + *D ext. and int.	- [2] + *S unif. [2] + *S unif. preantral [2] + *KOH/S unif. [2] + *D unif. [2]	- [3] - *S [3] + *KOH/S [3]	- [4] + *S [4]	+ [4] + *S [4]	+ [4] + *S [4]	+ [4] + *S [4]	+ [4] + *S [4]	+ [4] + *S [4]	+ [4] + *S [4]

			[2] + *D unif. preantral [2]									
RCA-I	- [1] + *D/S [1]	- [2] + *S unif. [2] + *KOH/S [2] + *D [2]	- [2] + *S unif. [2] + *KOH/S [2] + *D [2]	- [2] + *S unif. [2] + *KOH/S [2] + *D [2]	- [3] + *S [3] + *KOH/S [3]							
RCA-II						+ [4] + *S [4]	+ [4] + *S [4]	- [4] - *S [4]	- [4] - *S [4]	+ [4] + *S [4]	+ [4] + *S [4]	- [4] + *S [4]
SBA	- [1] + *S [1] + *KOH/S [1] + *D [1] + *D/S [1]	- [2] + *S unif. [2] + *KOH/S unif. [2] + *D [2]	- [2] + *S int. [2] + *S unif. preantral [2] + *KOH/S int. [2] + *KOH/S unif. preantral [2] + *D ext. and int. [2] + *D unif. preantral [2]	- [2] + *S unif. [2] + *KOH/S unif. [2] + *D ext. and int. [2] - *D preantral [2]	- [3] + *S [3] + *KOH/S [3]	- [4] + *S [4]	+ [4] + *S [4]	- [4] - *S [4]	- [4] + *S [4]	- [4] + *S [4]	- [4] + *S [4]	- [4] + *S [4]
DBA	- [1] - *S [1] - *KOH/S [1] - *D [1]	- [2] - *S [2] - *KOH/S [2] - *D [2]	- [2] - *S [2] - *KOH/S [2] - *D [2]	- [2] - *S [2] - *KOH/S [2] - *D [2]	- *KOH/S [3] - *D [3]							
UEA-I	- [1] - *S	- [2] - *S	- [2] - *S	- [2] - *S	- [3] -*S	- [4]	- [4]	- [4]	- [4]	- [4]	- [4]	- [4]

	[1] - *KOH/S [1] - *D [1]	[2] - *KOH/S [2] - *D [2]	[2] - *KOH/S [2] - *D [2]	[2] - *KOH/S [2] - *D [2]	[3] - *KOH/S [3] - *D [3]							
LTA	- [1] - *S [1] - *KOH/S [1] - *D [1]	- [2] - *S [2] - *KOH/S [2] - *D [2]	- [2] - *S [2] - *KOH/S [2] - *D [2]	- [2] - *S [2] - *KOH/S [2] - *D [2]	- [3]	- [4]	- [4]	- [4]	- [4]	- [4]	- [4]	- [4]
SNA						+ [4]	+ [4]	- [4]	- [4]	+ [4]	+ [4]	- [4]
GSA-II	- [1] - *S [1] - *KOH/S [1] - *D [1]	- [2] - *S [2] - *KOH/S [2] - *D [2]	- [2] - *S [2] - *KOH/S [2] - *D [2]	- [2] - *S [2] - *KOH/S [2] - *D [2]	- [3] - *S [3] - *KOH/S [3] - *D [3]							
GSA-IB4	- [1] - *S [1] + *KOH/S [1]	- [2] + *S unif. [2] + *KOH/S [2] + *D [2]	- [2] + *S unif. [2] + *KOH/S [2] + *D [2]	- [2] + *S unif. [2] + *KOH/S [2] + *D [2]	- [3] + *S [3] + *KOH/S [3]							
ECA					- [3] + *S [3] + *KOH/S [3]	- [4]	+ [4]	+ [4]	- [4]	- [4]	+ [4]	+ [4]
PSA						- [4]	+ [4]	- [4]	- [4]	+ [4]	+ [4]	- [4]

Analysis were carried out using sections of ovaries.
Positive binding: **+**; Negative binding: -
Binding localized in the external ZP surface: **ext**.; internal ZP surface: **int.**; medium ZP: **med.** or uniform binding: **unif.**
*: lectin binding procedures were preceded by different pre-treatments. Sialidase digestion: **S**; Saponification/sialidase digestion: **KOH/S**; Desulphation process: **D.**
Some references distinguish between primordial, primary, secondary, multilaminar and antral follicles.

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Table S3. Primers used for the RT-PCR amplification of *GAPDH* and *ZPAX* genes of Bennett's wallaby.

Gene	Forward 5′-3′	Reverse 5′-3′	Product size (bp)	GenBank
GAPDH	ATGCCCCAATGTTCGTGAT	ACAACAGATACATTGGGAGT	349	EF654515.1
ZPAX	CCAAGATGAGCTGAAGACTG	GCATCTTCAACATATCCGTG	720	Scaffold20091 Macropus eugenii
ZPAX	GATACAGTCTGAGGAGTGATG	CACGGATATGTTGAAGATGC	266	Scaffold20091 Macropus eugenii
ZPAX	GCAATGTAGACCAGAGCTG	GGACAAGTGATGGAGTATTC	241	Scaffold20091 Macropus eugenii