2. Detection of specific glycoproteins in oviductal secretions

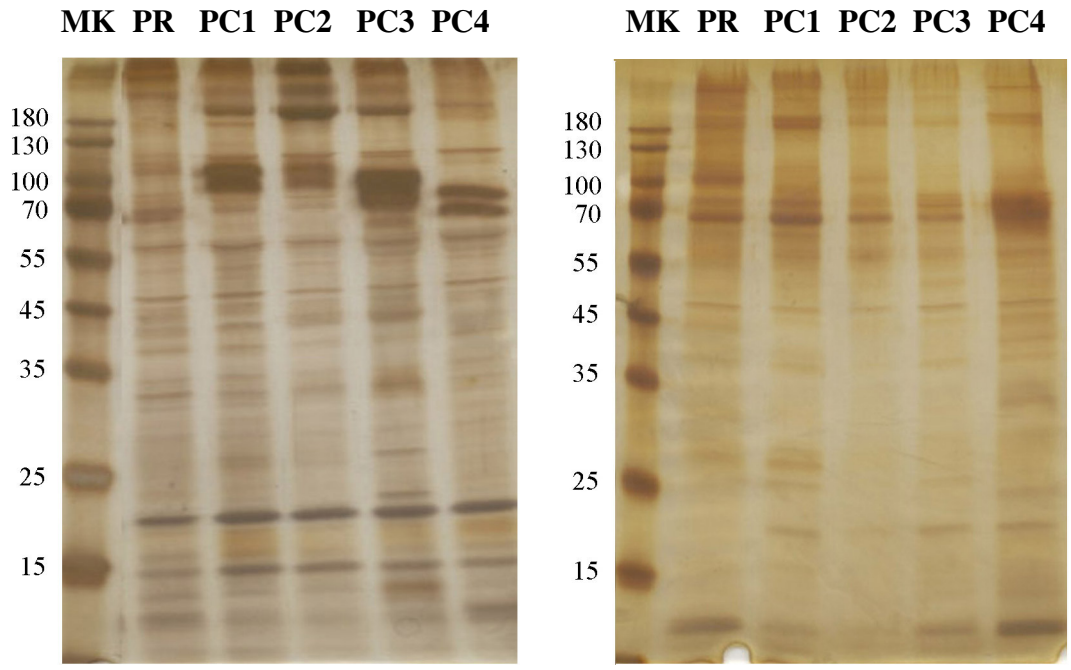
Further attempt was made to characterize specific carbohydrate moieties on the glycoproteins secreted by each portion of oviduct using lectin blotting. The same sets of lectins used for lectin histochemstry were also chosen as probes to detect specific sugar residues on each protein band. Initially, the oviductal secretory proteins were resolved by SDS-PAGE and subjected to silver staining. Protein profiles shown in Figure16 demonstrated that proteins taken from PR greatly differed from those taken from PC regions. Within the broad range of proteins in both PR and PC, the 80 and 150 kDa proteins appeared to be the major proteins found in the PR secretions while the 80, 150 and 180 kDa proteins were enriched in the secretions of all PC regions. Using lectins BSL-I and ConA, it was shown that these two lectins similarly recognized 4 major secretory proteins taken from various parts of oviducts (Figure 17). These major glycoproteins included slow migrating proteins with the molecular mass of about 130, 150 and 180 kDa in the PR, PC1 and PC2 regions as well as one medium migrating protein (65 kDa band) (Figure 17, panels A and B). Specifically, proteins flushed from the oviducts of non-breeding animals showed minimal reactivity with these two lectins (Figure 17, panels C, D and also other lectins shown in the next following Figures). Only the faint reactivity of the 58 kDa glycoprotein was detected by BSL-I (Figure 17, panel C). This indicated that despite the presence of considerable amount of proteins in oviductal lumen, most of these proteins were minimally glycosylated proteins. Of particular interest, the reactivity of lectins BSL-I and ConA on the secretory proteins collected from the progesterone administered animals was considerably higher than that of the seasonal breeders both in their intensity and the number of reactive protein bands. The highly intense 70 kDa protein was readily observed in the PC1 and PC2 glycoproteins and the additional moderately intense 55 kDa protein was detected in the PC3 and PC4 glycoproteins (Figure 17, panels E and F).

When the secretory proteins collected from the breeding period were subjected to LCA, the relatively similar protein set was recognized by LCA as with lectins BSL-I and ConA, namely, 150 and 180 kDa proteins (Figure 18, panel A). The slight difference was the presence of the 60 kDa reactive band in the proteins of PC3 and PC4. Two reversal events were noticed when the proteins of hormonal administered animals were subjected to LCA staining (Figure 18, panel E). While the staining intensity of the protein set in PR and PC1 significantly decreased, the intensity of both slow and medium migrating proteins, particularly the 60 kDa band, in PC2 to PC4 apparently increased. For RCA-I staining, secretory proteins collected from seasonal breeders showed a minimal reactivity with this lectin. Only the 150 kDa protein in PC2 and PC3 and the additional 180 kDa in PC3 were faintly reactive (Figure 18, panel B). A drastic difference in lectin reactivity was seen when the proteins of hormonal treated animals were subjected to the same lectin staining. A number of broad ranged proteins from 37 kDa to >180 kDa reactive with RCA-I were homogeneously distributed in the secretions of the PC1 to PC4, however, only a small number of proteins in the PR was restrictedly reactive with the most prominent one being a 150 kDa protein band (Figure 18, panel F).

Oviductal secretions collected during breeding period were moderately reactive with UEA-I and WGA lectins. For UEA-I (Figure 19, panel A), the major reactive protein bands in the PR and PC1 region were 150 and 180 kDa with a minor 80 kDa specifically to PR. Only a single reactive band of 60 kDa was found in the proteins of PC3 and PC4. Similar results were seen with WGA staining, however, a minor difference was the additional reactivity of a 130 kDa in the PC2-PC4 regions (Figure 19, panel B). Out of the expectation, the inhibitory effect of progesterone treatment on the secretions of UEA-I and WGA reactive proteins was reversal to its enhancing effect on the secretions of other lectins' reactive proteins mentioned above. Between the two lectins used, there was only a single band of a WGA reactive protein (~120 kDa) being secreted by the oviducts of the hormonal administrated animals (Figure 19, panel F).

Figure 16 **The oviductal secretory proteins resolved by SDS-PAGE and subjected to silver staining.**

Protein profiles demonstrated that proteins taken from PR greatly differed from those taken from PC regions. A = breeding period, B = non-breeding period, C = hormonal administration period.


MK PR PC1 PC2 PC3 PC4
180
130
100
70
55
45
35
25
15
A
MK PR PC1 PC2 PC3 PC4
180
130
100
70
55
45
35
25
15
B

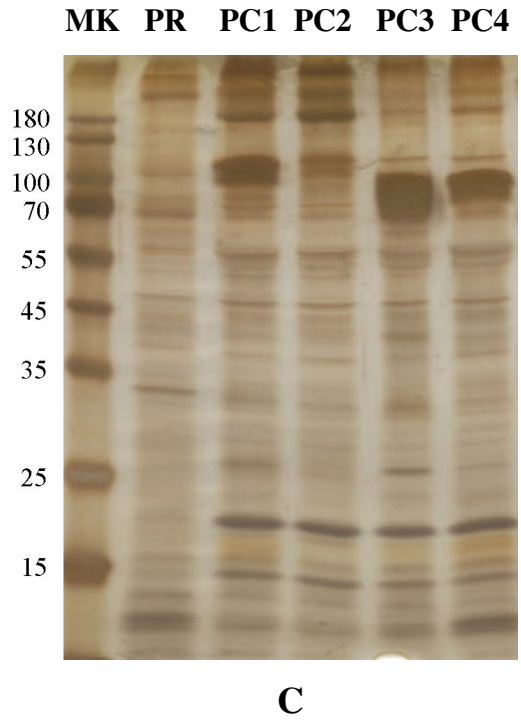

MK PR PC1 PC2 PC3 PC4
180
130
100
70
55
45
35
25
15
C

Figure 17 **Lectin blotting with BSL-I (panels A, B and C) and ConA (panels D, E and F) of the secretory proteins taken from all parts of oviducts in various conditions.**

Note the higher intensity of lectin staining in the secretory proteins of the animals treated by progesterone. Standard molecular mass marker is shown on the left of each panel. A and B = breeding period, C and D = non-breeding period, E and F = hormonal administration period.

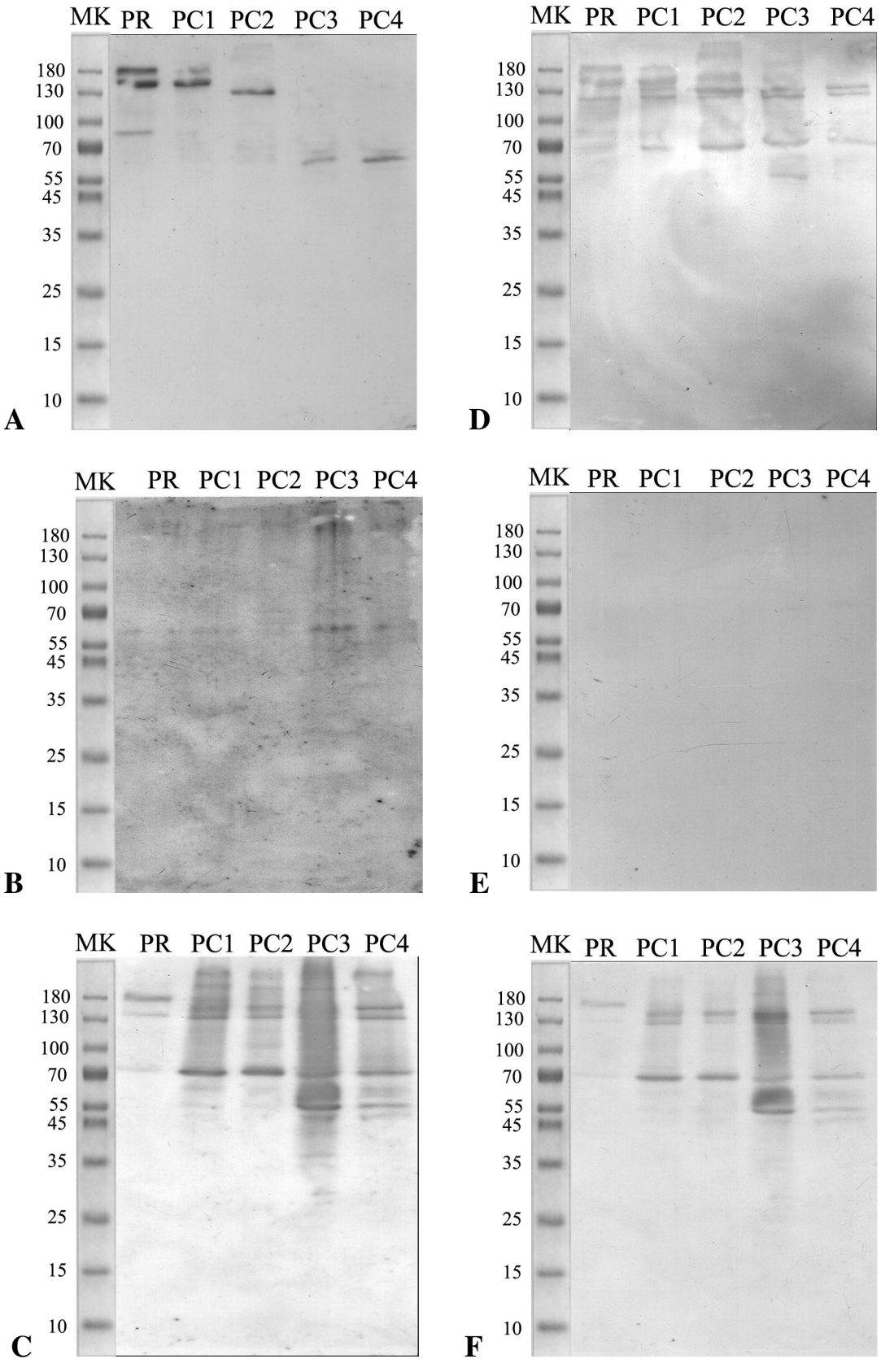

MK PR PC1 PC2 PC3 PC4
180
130
100
70
55
45
35
25
15
10
A
D
B
E
C
F
BSL-I
ConA

Figure 18 **Lectin blotting with LCA (panels A, B and C) and RCA-I (panels D, E and F) of the secretory proteins taken from all parts of oviducts in various conditions.**

Note the higher intensity of lectin staining in the secretory proteins of the animals treated by progesterone. Standard molecular mass marker is shown on the left of each panel. A and B = breeding period, C and D = non-breeding period, E and F = hormonal administration.

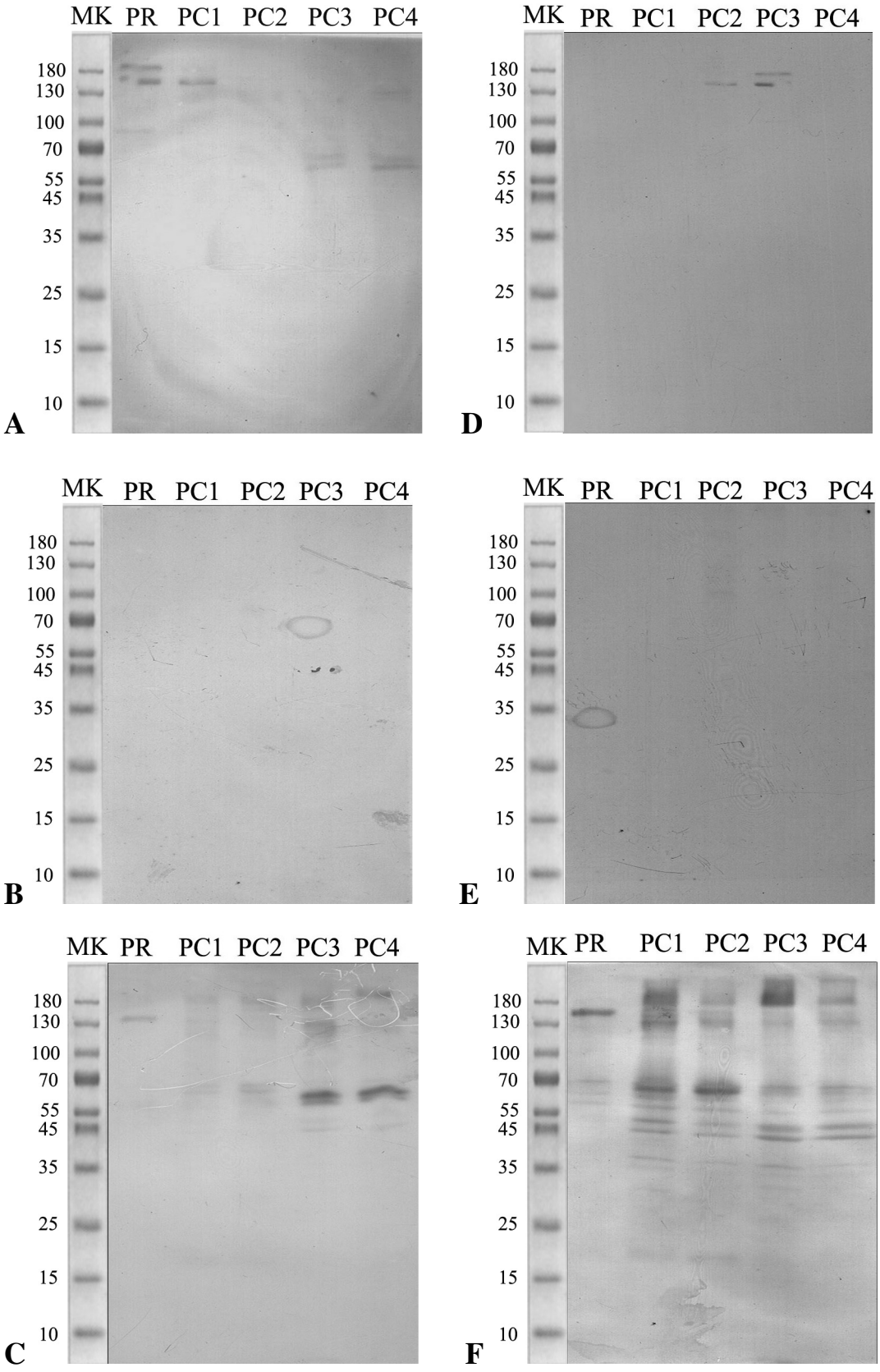
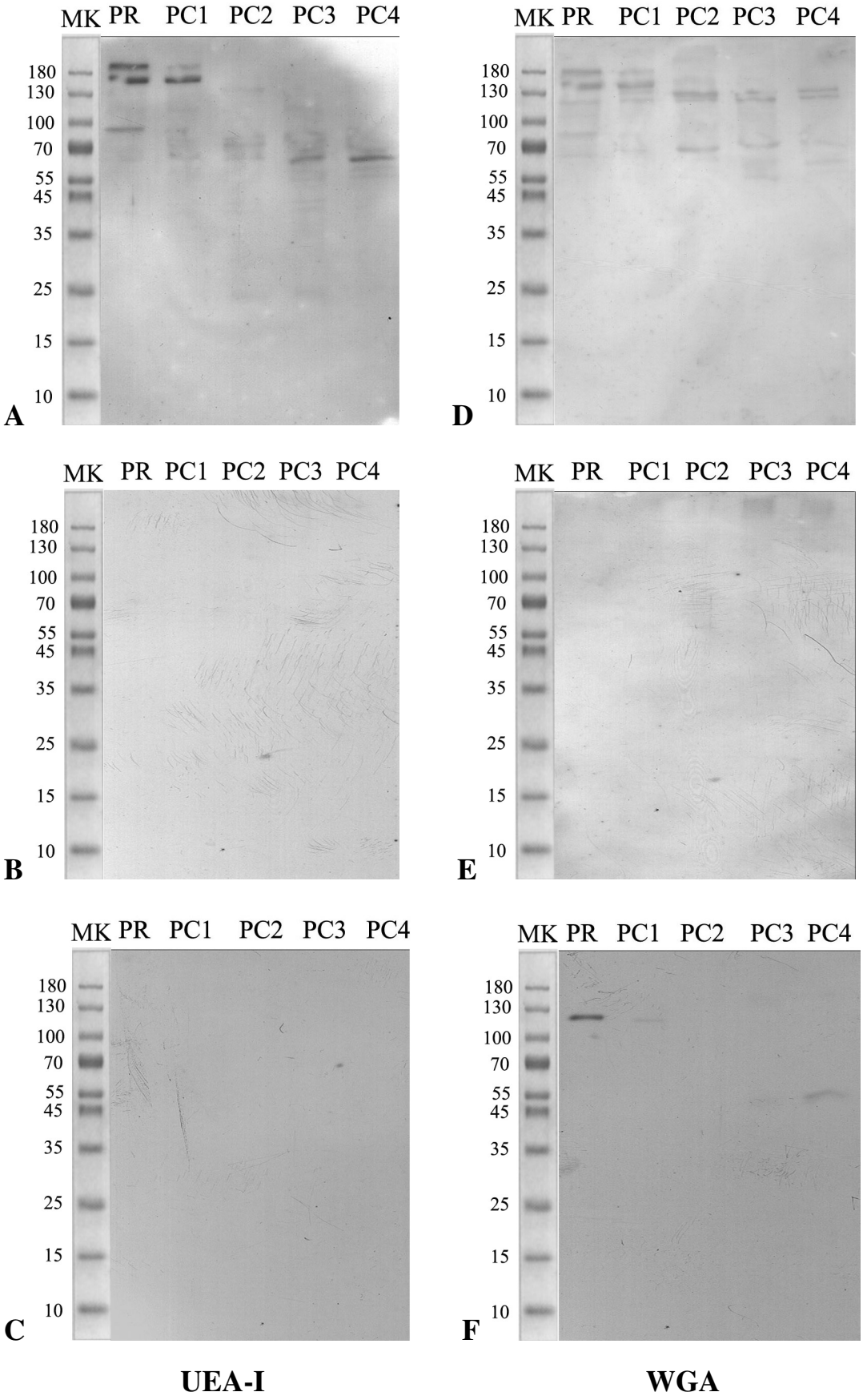

MK PR PC1 PC2 PC3 PC4
180
130
100
70
55
45
35
25
15
10
A
D
B
E
C
F
LCA
RCA-I

Figure 19 **Lectin blotting with UEA-I (panels A, B and C) and WGA (panels D, E and F) of the secretory proteins taken from all parts of oviducts in various conditions.**

Note the higher intensity of lectin staining in the secretory proteins of the animals treated by progesterone. Standard molecular mass marker is shown on the left of each panel. A and B = breeding period, C and D = non-breeding period, E and F = hormonal administration**.**


MK PR PC1 PC2 PC3 PC4
180
130
100
70
55
45
35
25
15
10
A
D
B
E
C
F
UEA-I
WGA