

Supplementary materials to Souri et al. “Increase in the plasma levels of protein Z-dependent protease inhibitor in normal pregnancies but not in non-pregnant patients with unexplained recurrent miscarriage” (Thromb Haemost 2012; 107.3)

Fig. S1. Correlation analysis between age and plasma ZPI or PZ levels in normal Japanese (A) and German (B) controls. No statistically significant correlations were obtained for both normal Japanese (n=42) and German (n=64) controls and females with normal pregnancy ($R^2=0.001$ to 0.08 , $P=0.076$ to 0.90).

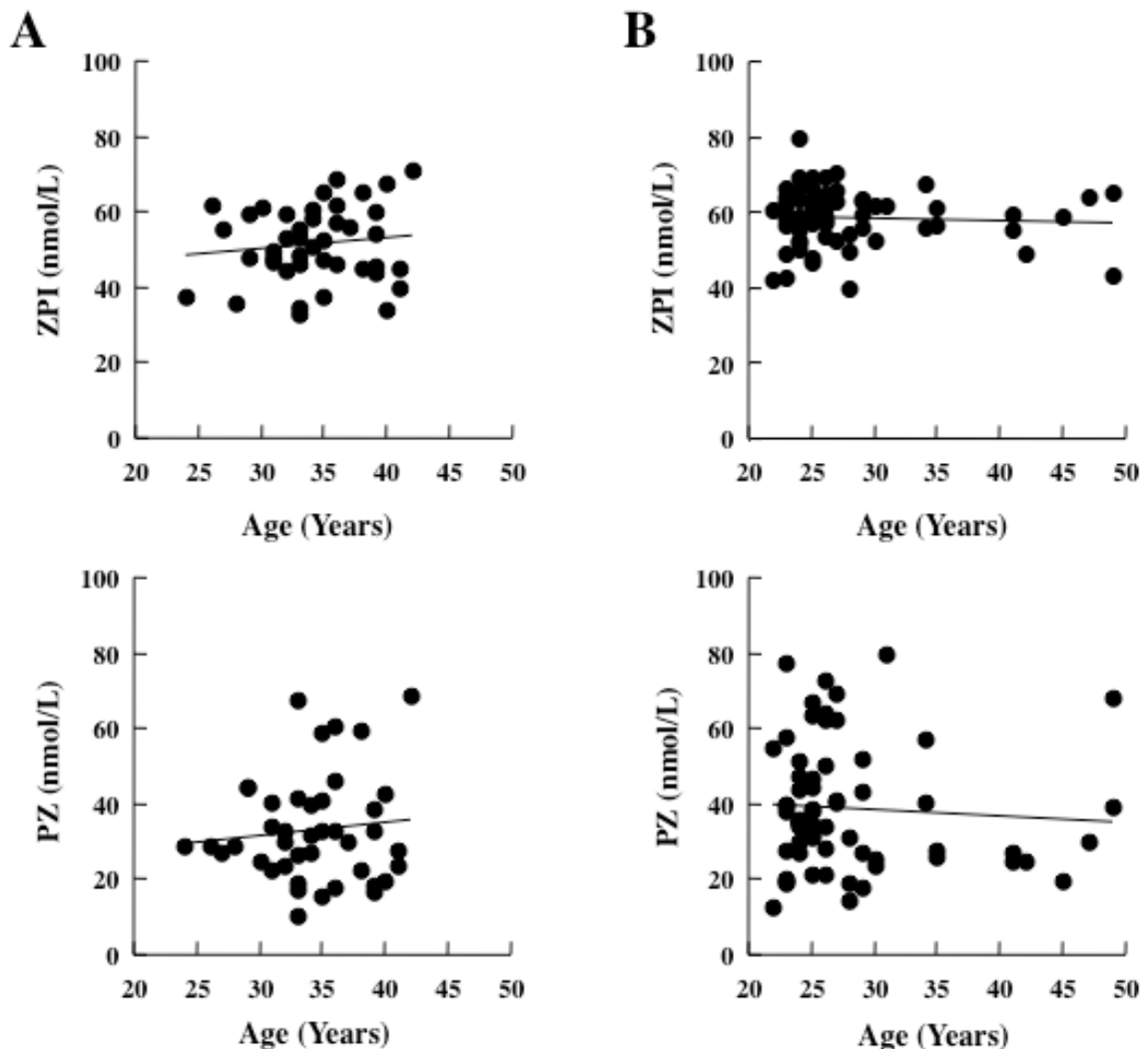


Fig. S2. Prospective longitudinal measurements of ZPI (A) and PZ (B) concentrations during normal pregnancy.

32 volunteers participated in the longitudinal study, and their blood samples were available at all time points. Data are presented as the means of more than triplicates. Twenty-nine and 31 out of the 32 participants demonstrated steady increases in ZPI and PZ levels, respectively.

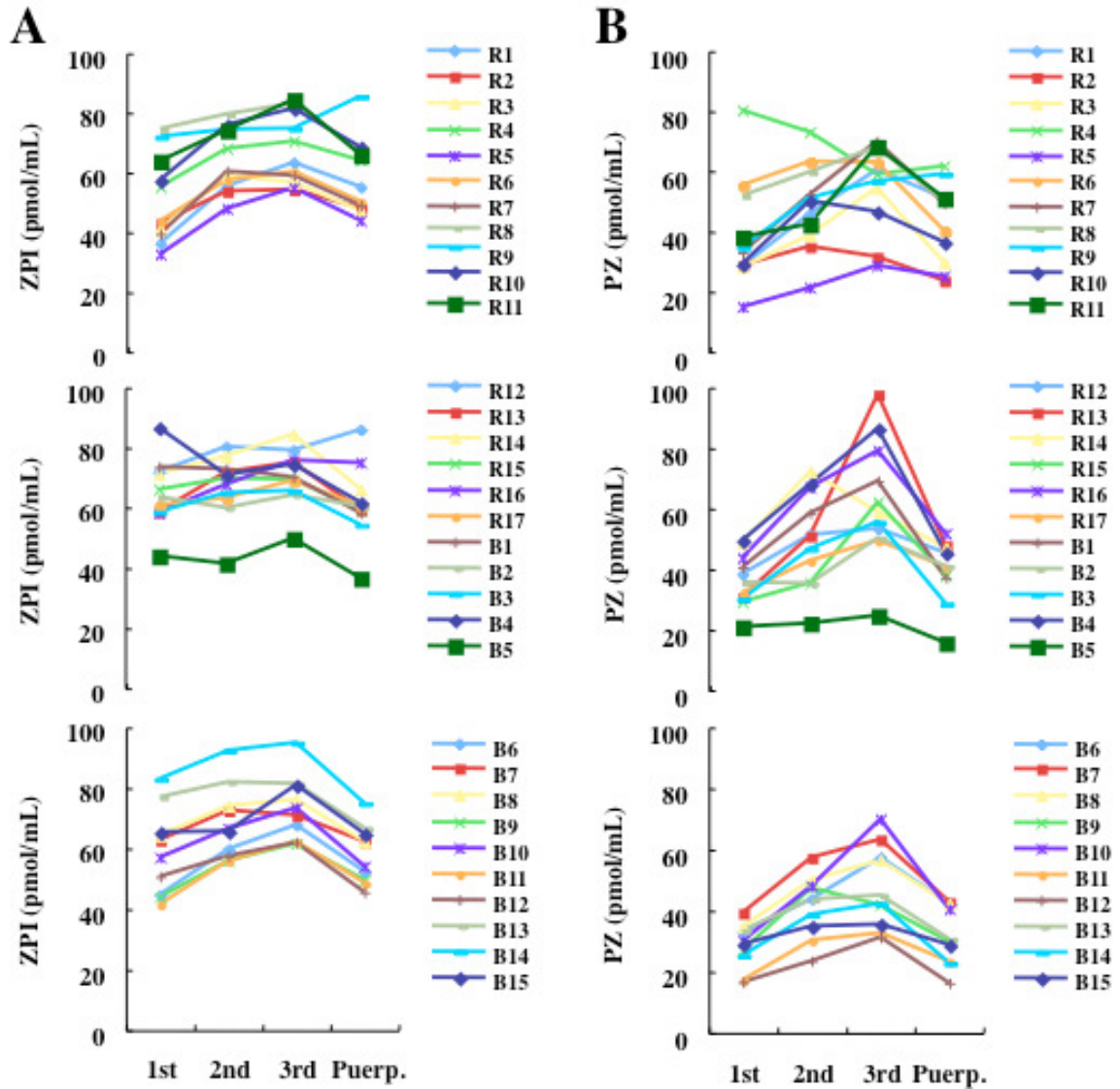


Fig. S3. The relationship between ZPI and PZ levels in Japanese women with normal pregnancy (A) and in RM cases (B)

A. A statistically significant correlation was obtained for Japanese women with normal pregnancy of all periods (n=32) ($R^2=0.27$, $P<0.001$; solid line) as well as during the 2nd trimester and Puerp. ($R^2=0.23$, $P=0.005$ and $R^2=0.31$, $P=0.001$, respectively). A trend toward a positive correlation was also observed during the 1st and 3rd trimesters ($R^2=0.12$, $P=0.052$ and $R^2=0.11$, $P=0.059$, respectively). Gray, black, red, and blue circles and lines represent the 1st, 2nd, 3rd and puerperium periods, respectively. **B.** A good correlation was found between ZPI and PZ in patients with RM (n=134) ($R^2=0.34$, $P<0.0001$; solid circles & line) or in non-pregnant individuals (n=42) ($R^2=0.28$, $P<0.001$; open circles and broken line) as shown in Fig. 2A.

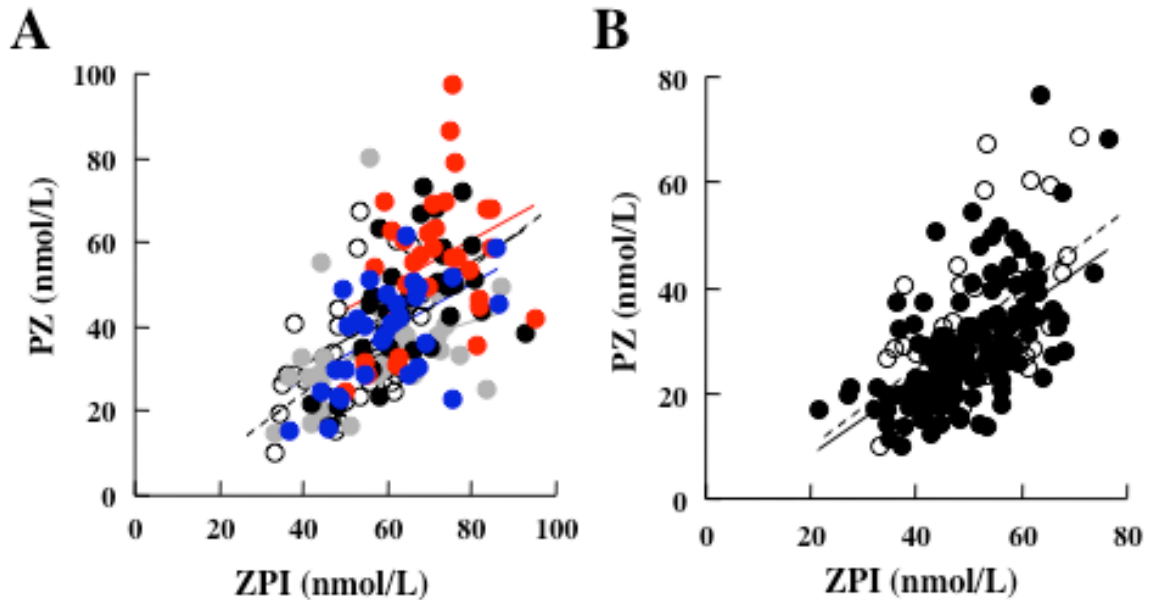


Fig. S4. Plasma FX (A) and its relationships between ZPI (B) and PZ (C) levels in Japanese women with normal pregnancy and in non-pregnant controls.

A. Plasma FX was measured by a clotting assay using FX-deficient plasma. Data are presented as described in Fig.1. Statistically significant increases in FX were observed between the 2nd and 3rd gestational periods of women with normal pregnancy versus non-pregnant controls, the pattern of which closely resembles that of PZ (Fig. 3B). **, $P<0.01$. **B & C.** Although good correlations were found between FX and ZPI ($R^2=0.18$, $P=0.005$; long broken line in **B**) and PZ ($R^2=0.37$, $P<0.001$; long broken line in **C**) in non-pregnant controls, there were no significant correlations between FX and ZPI ($R^2=0.01-0.084$, $P=0.11-0.59$; short solid lines) or PZ ($R^2=0.009-0.046$, $P=0.22-0.71$; short solid lines) for any gestational period of women with normal pregnancy except for ZPI during the puerperium period ($R^2=0.13$, $P=0.043$; blue circles and solid line with # in **B**). Gray, black, red, and blue circles and lines represent the 1st, 2nd, 3rd and puerperium periods, respectively.

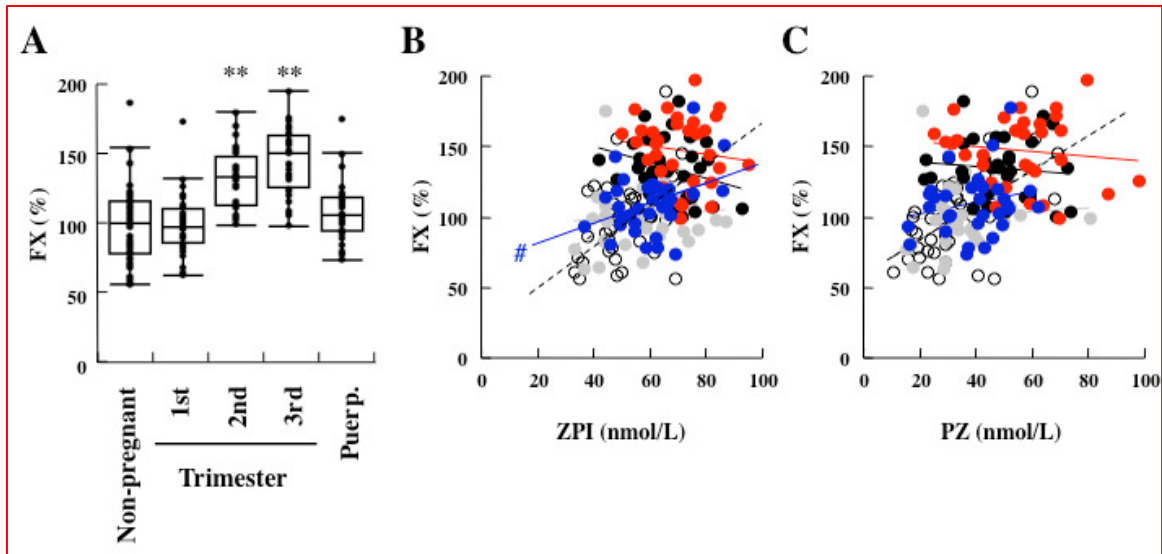


Fig. S5. Plasma FX (A) and its relationships between ZPI (B) and PZ (C) levels in patients with RM.

A. Data are presented as described in Fig. S4. No statistically significant difference was observed for FX between patients with RM (n=134) versus non-pregnant controls (n=42) ($P=0.23$). B & C. There were significant positive relations for FX and ZPI ($R^2=0.18$, $P=0.015$) and PZ ($R^2=0.37$, $P<0.001$) among non-pregnant controls (open circles, broken lines), and for ZPI ($R^2=0.047$, $P=0.015$) but not for PZ ($R^2<0.001$, $P=0.84$) in RM cases (solid circles & lines).

