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Detection of MMP-9, MMP-1 and TIMP-1 in the Lung of Developing Mouse after Prenatal Administration of Vitamin A

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Matrix metalloproteinases (MMPs) are a family of proteolytic enzymes that together with their tissue inhibitors (TIMPs) are important modulators of normal lung development and harmful mediators of lung damage. Several studies support the role for an imbalance of MMPs and TIMPs homeostasis in the pathogenesis of paediatric lung failure [2]. It was verified that MMP-9 and MMP-9/TIMP-1 ratio increases in the broncho-alveolar lavage of patients with bronchopulmonary dyspasia (BPD) [3]. Maternal administration of vitamin A, once the period of retinoid-induced teratogenesis is over, results in an enhancement of lung foetal organogenesis, an increase in the lung's elastic fibres, and an increase in VEGF pulmonary and plasma levels [4].

The ontogeny of lung's MMP-9, MMP-1, and TIMP-1 after prenatal administration of vitamin A was determined. Pregnant mice were subjected to subcutaneous administration of vitamin A on the 12^{th} gestational day. Lungs were collected daily from the 15^{th} gestational day till the day of birth, and processed for routine immunohistochemistry. The degree of positive staining was evaluated by semi-quantitative scoring on a scale of 0 (negative cases) to 3 for intensity (I) and distribution (D). Tissues with I x D less than or equal to 3 were considered weakly positive, and those with I x D greater than 3 were designated strongly positive. Immunohischemical scores were assessed by the $\chi 2$ test. Statistical significance was set at $p<0.05$.

In the lung of control foetuses MMP-9 expression is higher at the 16^{th} gestational day. In vitamin A treated foetuses there is a decrease in MMP-9 expression at this day, but a progressive enhancement of it's expression can be observed. MMP-1 expression is weak or absent at the pseudoglandular stage, strong at first day of the canalicular stage, but decreases at the17^{th} gestational day, and increases again at the saccular stage. TIMP-1 expression is high during all stages of lung development. Our findings suggest that vitamin A modulates MMP-9 during foetal lung development and hence, the MMP-9/TIMP-1 ratio (Fig. 1), with a marked anti-proteolytic profile at the 16^{th} gestational day ($p=0.046$), and a proteolytic profile at the last gestational day. These changes could be important to lung maturation and may help to reduce the risk of BPD.

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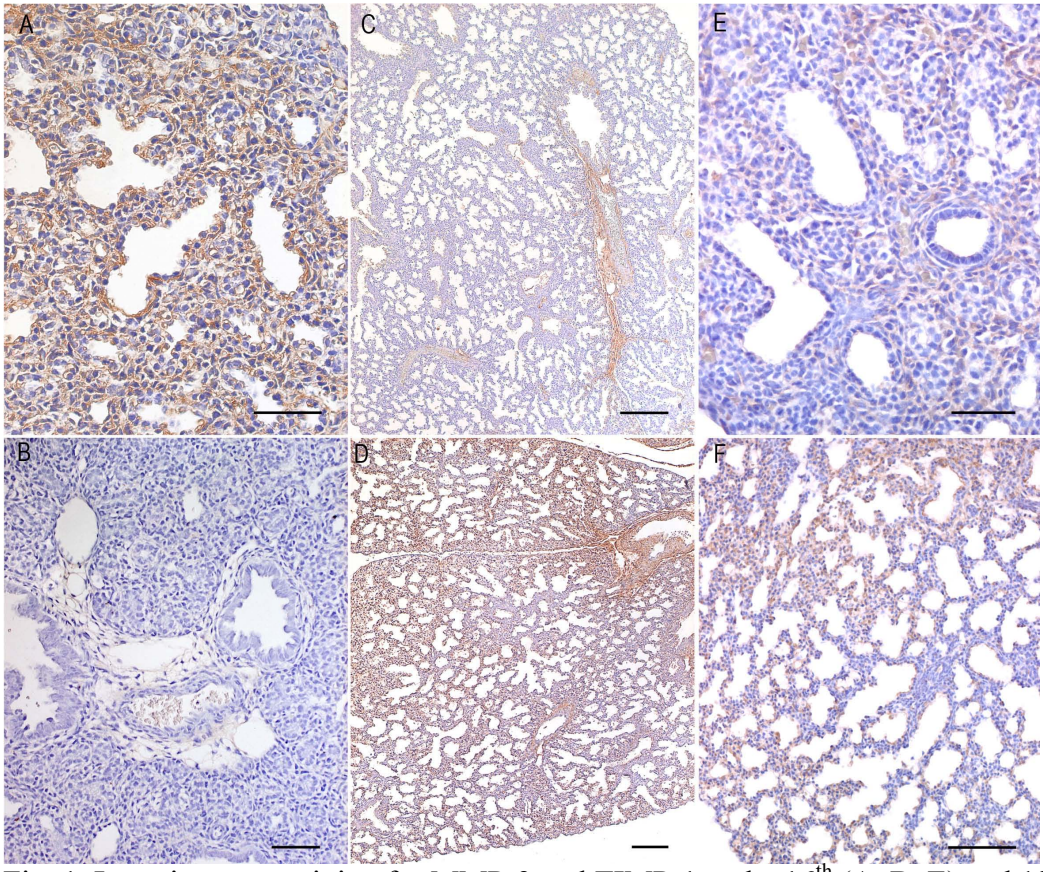

Fig. 1. Lung immunostaining for MMP-9 and TIMP-1 at the 16th (A, B, E) and 18th (C, D, F) gestational days: A and C. MMP-9 expression in control foetuses; B and D. MMP-9 expression in treated foetuses. Scale bars: A and F =100 µm; B and C = 150 µm; D = 300 µm;E = 50 µm.

Table 1: MMP-9 immunostaining scores (Intensity x Distribution).

	MMP-9 Immunostaining Scores							
	Control				Vitamin A			
Gestational days	None	Weak	Moderate	Strong	None	Weak	Moderate	Strong
15	0	0	4	0	0	0	4	0
16	0	0	3	1	1	3*	0	0
17	0	1	3	0	0	1	3	0
18	0	3	1	0	0	0	3	1
0^{+}	0	1	3	2	0	0	0	4

$^{+}$ day of birth; * p=0.046 (vs control)

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