Cite this as: Zhen DAI, Rong WU, Yi-cheng ZHAO, Kan-kan WANG, Yong-ye HUANG, Xin YANG, Zi-cong XIE, Chang-chun TU, Hong-sheng OUYANG, Tie-dong WANG, Da-xin PANG, 2014. Early lethality of shRNA-transgenic pigs due to saturation of microRNA pathways. *Journal of Zhejiang University-Science B (Biomedicine & Biotechnology)*,15(5):466-473 [doi:10.1631/jzus.B1400001]

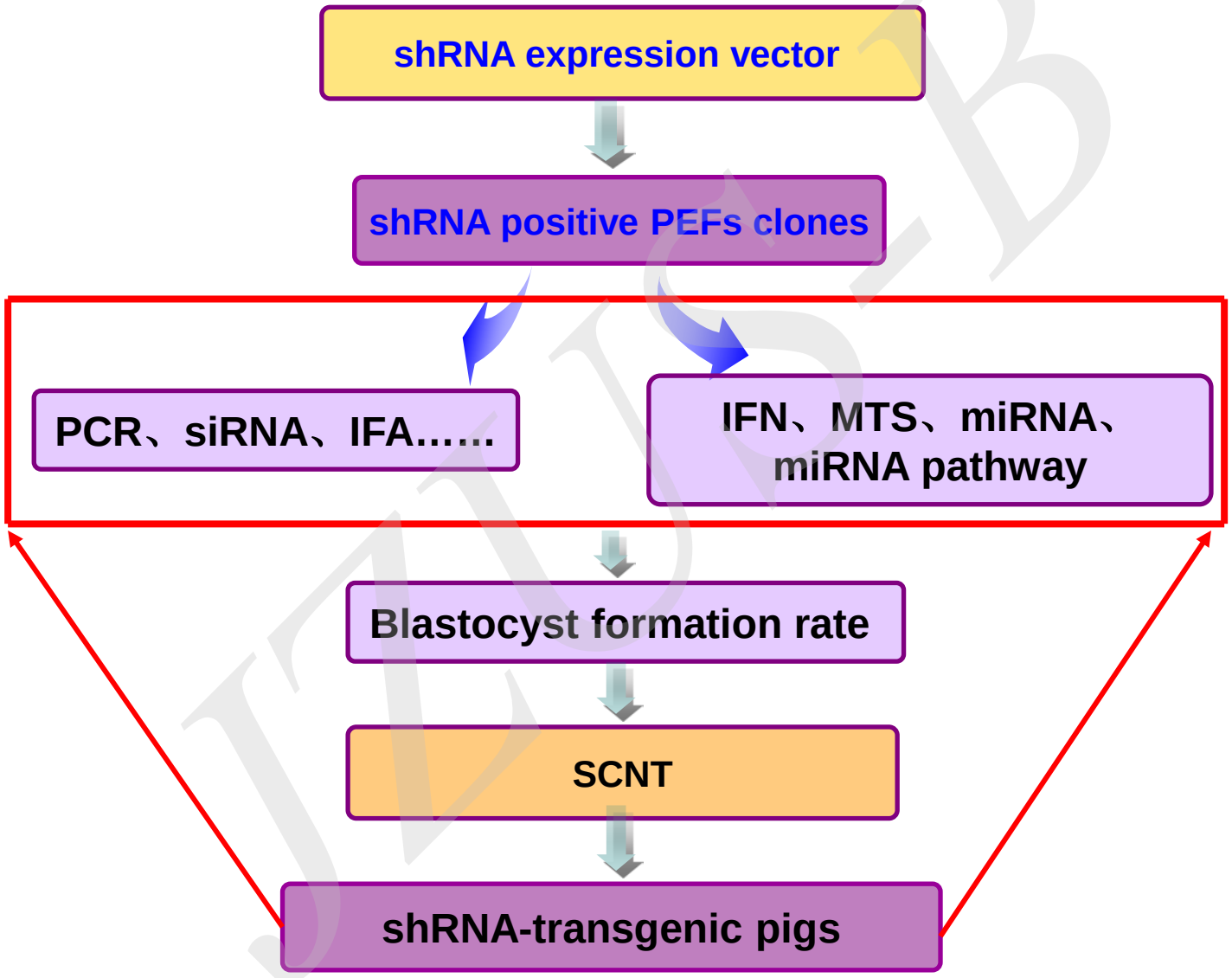
Early lethality of shRNA-transgenic pigs due to saturation of microRNA pathways

Key words: MicroRNA pathway, shRNA-transgenic pigs, Classical swine fever virus (CSFV), Blastocyst formation, Early lethality

- Our previous studies found that a low survival rate and early lethality were observed in shRNA-transgenic pigs compared with other transgenic pigs when we attempted to produce shRNA-transgenic pigs with anti-classical swine fever virus (CSFV) capacity.
- Several researchers reported hepatoxicities and fatalities induced by ectopic RNAi triggers when they attempted to intravenously inject adeno-associated virus (AAV)-mediated shRNA vectors into mouse models (Ahn et al., 2011; Borel et al., 2011; Martin et al., 2011) which seriously hindered therapeutic RNAi. On the other hand, RNAi technology has been widely applied to inhibit viruses in vitro , including human immunodeficiency virus, hepatitis c virus, poliovirus, foot-and-mouth disease virus, porcine transmissible gastroenteritisvirus, etc. Recently, Maillard et al. (2013) demonstrated that antiviral RNAi operates in mammalian cells. However, few reports have been published regarding the production of transgenic animals resistant to viruses by RNAi. Cell reprogramming that occurs in the process of somatic cell nu clear transfer (SCNT) may convert exogenous shRNA cassettes into endogenous microRNAs (miRNAs). Whether in vivo toxicities induced by intravenously injected exogenous small interfering RNA (siRNA)/ shRNA would occur in shRNA-transgenic animals remains controversial.

- **Thus, we investigated the the feasibility of inhibiting CSFV replication by shRNA and shRNA induced adverse effects in vitro and in vivo.**

Experiment procedures



- In our study, CSFV could be effectively inhibited in shRNA-positive clonal cells and tail tip fibroblasts of shRNA-transgenic pigs. We found that shRNAs led to the induction of interferon (IFN)-responsive genes and abnormalities in endogenous miRNAs and their processing enzymes in shRNA-positive clonal. Saturation of the miRNA pathway and altered endogenous miRNA levels were also discovered in the transgenic pig livers, which explained the fatality of the shRNA-transgenic pigs in our experiment. Finally, we investigated the effects of shRNAs on the development of SCNT embryos by measuring the blastocyst formation rate. These results show that shRNA causes adverse effects in vitro and in vivo and shRNA-induced disruption of the endogenous miRNA pathway may lead to the early lethality of shRNA-transgenic pigs. We firstly report abnormalities of the miRNA pathway in shRNA-transgenic animals, which may explain the early lethality of shRNA-transgenic pigs and have important implicat ions for shRNA-transgenic animal preparation.