



Article

# Integrating Transcriptomic and ChIP-Seq Reveals Important Regulatory Regions Modulating Gene Expression in Myometrium during Implantation in Pigs

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Abstract: The myometrium is the outer layer of the uterus. Its contraction and steroidogenic activities are required for embryo implantation. However, the molecular mechanisms underlying its functions remain unknown in pigs. The myometrium includes the inner circular muscle (CM) and the outer longitudinal muscle (LM) layers. In this study, we collected the CM and LM samples from the mesometrial side (named M) of the uterus on days 12 (pre-implantation stage) and 15 (implantation stage) of pregnancy and day 15 of the estrous cycle. The transcriptomic results revealed distinct differences between the uterine CM and LM layers in early pregnancy: the genes expressed in the LM layer were mainly related to contraction pathways, whereas the transcriptional signatures in the CM layer on day 15 of pregnancy were primarily involved in the immune response processes. Subsequent comparisons in the CM layer between pregnant and cyclic gilts show that the transcriptional signatures of the CM layer are implantation-dependent. Next, we investigated the genome-wide profiling of histone H3 lysine 27 acetylation (H3K27ac) and histone H3 lysine 4 trimethylation (HJK4me3) in pig uterine CM and LM layers. The genomic regions that had transcriptional activity and were associated with the expression of genes in the two layers were characterized. Taken together, the regulatory regions identified in the study may contribute to modulating the gene expression in pig uterine CM and LM layers during implantation.

Keywords: pigs; implantation; myometrium; regulatory regions; H3K27ac; H3K4me3

Citationi Wang, W.; Can, C.; 22hang, B.; Winng, F.; Dong, D.; Can, J.; Li, H.; Yu, M. Integrating Esamoriphomic and CMF-Seq Reviols Important Regulatory Registra Modulating Gene Expression in Myometrians during Implantation in Pigs. Biomelocales 2023, 13, 45. https://doi.org/ 10339005eem13000045

Academic Editors: Mirguing Liand Charppy Benegions

Received: 12-October 2022 Barrised: 17 Occomber 2022 Accepted: 22 December 2022 Published: 24 December 2022



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#### 1. Introduction

Litter size is one of the most economic traits in swine production. Multiple factors and processes determine the pregnancy outcome. Success in implantation and embryo development is required to achieve a large litter size. The uterus plays a fundamental role in implantation and embryo development during pregnancy. Structurally, the uterus consists of three layers: the endometrium, myometrium, and perimetrium [1]. An endometrium is where the embryo implants and resides during pregnancy. Thus, its role in embryo implantation has been studied intensively and well-determined [2–6]. The myometrium consists of two layers of smooth muscle. Its primary function is to generate uterine contraction and steroidogenic activity, which are also required for implantation [7–9].

Much progress has been made toward understanding the physiological mechanisms of myometrial activity in implantation. In polytocous species such as rabbits, mice, rats, and pigs, it is ideal that embryos can implant evenly along the uterine horns. Thus, intrauterine embryo spacing is a common event during pre-implantation. The mechanical force generated by the myometrial activity of the uterus is essential in the migration of embryos in the uterus [9–11]. In addition, the myometrium also plays an endocrine role

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