Dissecting the role of *WBP1* in preimplantation embryonic development: Insights into hippo signaling and trophectoderm formation in the bovine

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A SNP in WW binding domain protein (*WBP1*) has been linked to cow conception rate, a fertility trait in dairy cattle, and embryonic development in vitro. WBP1 interacts with the WW domains and serves as a mediator of protein-protein interactions. In the embryo, the Hippo signaling pathway is composed of many proteins that contain WW domains, including the Yes-associated protein (YAP) which has been associated with trophectoderm (TE) formation. Previous research from our group has shown that when the expression of *WBP1* is reduced, blastocyst rate decreases and so does its number of trophectoderm cells. This suggests that WBP1 may be involved in trophectoderm differentiation, however, the exact mechanism of action of WBP1 in the embryo is still unknown. The objective of this study was to determine how the functional ablation of WBP1 affects preimplantation embryonic development. To test this, a CRISPR-CAS9 system was used to ablate *WBP1*. Briefly, two guides spanning intron 1 and exon 2 were annealed to a tracer and to CAS9mRNA to form a solution of 62.5 ng/ul of each guide and 125 ng/ul of CAS9mRNA. The complex of guideRNA-CAS9 was electroporated into presumptive zygotes 10 hours post-insemination for treated embryos and just Cas9mRNA for controls. Zygotes were cultured in SOF-BEII media at 38.5°C with 5%CO2 and 5%O2. Cleavage was measured at day 3 and blastocyst at day 7.5 of development. To assess trophectoderm formation, immunolocalization of CDX2, phosphorylated YAP, and YAP translocation to the nucleus was measured at the 8-16 cell and morula stages. Images were analyzed using ImageJ v1.44. Cleavage was not different ($P = 0.1282$) between *WBP1*-null and control embryos (LSMEANS $\pm$ SEM: 74.84 $\pm$ 3.27% and 67.44 $\pm$ 3.64%, respectively). Control embryos had a blastocyst rate of 31.60 + 3.4% (LSMEANS $\pm$SEM), however, WBP1-null embryos did not produce blastocyst, and embryos were arrested at the morula stage ($P < 0.0001$). At the 8-16 cell stage, there were fewer ($P = 0.0028$) nuclear YAP-positive cells on *WBP1*-null embryos (53.13 $\pm$ 6.32% of total cells), compared to controls (82.65 $\pm$ 3.83% of total cells). In addition, the mean intensity of phosphorylated YAP was higher ($P = 0.0246$) in *WBP1*-null embryos (40.06 $\pm$ 3.21) compared to controls (24.76 $\pm$ 2.78). There was no difference ($P = 0.866$) in the number of CDX2-positive cells between groups at this stage. In contrast, at the morula stage, *WBP1*-null embryos had fewer ($P = 0.017$) cells with nuclear YAP (56 $\pm$ 3.75%), compared to control embryos (81.87 $\pm$ 2.95%). In addition, *WBP1*-null morulas had a reduced ($P = 0.0195$) number of CDX2-positive cells (49.14% $\pm$ 3.78%) than control (64.33 $\pm$ 3.66%). These results indicate that WBP1 has an important role in the hippo signaling pathway by regulating YAP. The absence of WBP1 causes increased YAP phosphorylation and degradation in the cytoplasm impairing TE differentiation which results in embryonic arrest before the blastocyst stage. Further research is required to elucidate if WBP1 acts in other proteins from the hippo signaling or how it regulates YAP function. Funding provided by USDA-AFRI 2022-6701538938.