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Revealing the mechanism of fiber promoting sow embryo implantation by altering the abundance of uterine fluid proteins: A proteomic perspective

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ABSTRACT

Many studies have shown that fiber in the diet plays an important role in improving the reproductive performance of sows, but there is rarely research on the impact of fiber on early embryo implantation. This study used 4D-Label free technology to identify and analyze the effect of the fiber composition in the diet on the protein in the early pregnancy uterine fluid (UF) of sows. The results indicate that ratio of insoluble fibers to soluble fibers (ISF/SF) 4.89 can increase the concentration of progesterone (PROG) and reduce tumor necrosis factorα (TNF-α) concentration in sow UF. In addition, through 4D-Label free, we identified a total of 4248 proteins, 38 proteins abundance upregulated and 283 proteins abundance downregulated in UF. Through enrichment analysis of these differential abundance proteins (DAPs), it was found that these differential proteins are mainly related to the docking of extracellular vesicles, vesicular transport, inflammatory response, and insulin resistance. Therefore, the results of this study reveal the possible mechanism by which fiber improves the reproductive performance of sows, laying a theoretical foundation for future research on the effects of diet on reproduction.

Significance: This study demonstrates the importance of dietary fiber for early embryo implantation in sows. The effect of dietary ISF/SF on early embryo implantation in sows was elucidated from a proteomic perspective through 4D-Label free technology. This study not only has significant implications for improving sow reproductive efficiency, but also provides important theoretical references for studying early miscarriage and reproductive nutrition in human pregnancy.

1. Introduction

Due to the similarity in body structure, physiological characteristics, and genome between pigs and humans, pigs have become a research model for human diseases [1,2]. During sow gestation, approximately 30% of embryos are lost during embryo implantation in early gestation, which is mainly due to abnormal maternal-fetal communication [3]. In the early stages of pregnancy, Uterine Fluid (UF) is the only liquid medium that connects free embryos and the endometrium, it provides the ideal environment for embryo implantation and is crucial for information transmission between the mother and embryo, as well as embryo implantation [4]. Studies have indicated the connection between the embryo and the endometrium is in part due to the proliferation and migration of embryonic trophoblast cells to the endometrium, thereby establishing a physiological connection between the mother and the embryo [5,6]. Recent studies have shown that exosomes in UF play an important role in regulating the proliferation and migration of embryonic trophoblast cells [7]. In addition, some researchers have found that Leukemia inhibitor factor (LIF) in UF is significant for embryo implantation, and the loss of LIF gene can lead to embryo implantation failure and infertility in mice [8,9]. The ion balance in UF also affect the implantation process of mouse embryos [10]. Therefore, UF is crucial for embryo implantation in the early pregnant stage.

Fiber is usually considered a nutrient that is not easily digestible, and is divided into soluble fiber (SF) and insoluble fiber (ISF) based on its ability to dissolve in water. Increasing the proportion of soluble fiber in

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the diet can improve the gut microbiota and the content of short chain fatty acids (SCFAs) produced by fermentation in sows, protect against inflammation and oxidative stress caused by high-energy diets, and reduce the proportion of intrauterine growth retardation (IUGR) piglets and improve the uniformity of piglets [11,12]. Our research group previously found that, when the ISF/SF ratio in gestation diet were 3.89 and 5.59, it promoted the growth of first parity piglet growth and development, and increased the average litter size and litter weight of the four successive parities [13]. Therefore, an appropriate ISF/SF in the diet can improve the reproductive performance of sows. He et al. showed that UF protein plays an important role in embryo implantation in early gestation of sows [14]. Previous studies have found that maternal dietary habits can alter the UF environment, which may affect embryo quality or related gene expression [15,16]. However, these studies mainly focus on the effects of dietary protein on amino acids in UF during pregnancy, there is no explanation from the perspective of UF proteomics. In view of the important role of dietary fiber in improving the reproductive performance of sows, we fed sows with two different ISF/SF diets during early gestation to investigate the effect of dietary fiber on UF protein abundance at day 19 of gestation. Therefore, this study is the first to reveal the mechanism of fiber in improving sow reproductive performance from the perspective of UF proteomics, and provides a theoretical basis for the application of fiber in sow diets and human reproductive nutrition.

2. Material and methods

2.1. Animals and experimental design

The experiment was conducted in the teaching and research base of the Animal Nutrition Institute of Sichuan Agricultural University (Yaan, China), and all the experimental procedures and methods were approved by the Animal Management Committee of Sichuan Agricultural University (ethical approval code: SICAU2021214037).

This study selected 20 Landrace × Yorkshire (LY) primiparous sows with body weight of 144.95 ± 1.64 kg and backfat of 19.98 ± 0.34 mm, randomly divided into two groups: control group (CON, basal diet ISF/SF = 7.47), treatment group (TRE, basal diet+11 g/kg inulin, ISF/SF = 4.96), each group has 10 replicates, with 1 sow per replicate. TRE supplemented with inulin (Beneo-Orafti, Belgium) to adjust the ISF/SF. The ISF/SF refers to the previous study of our research group. When the ISF/SF of pregnancy diet was 3.89 and 5.59, the reproductive performance of primiparous sows could be improved [13], and inulin is often added to animal diets as a SF [17,18]. Therefore, we used inulin to adjust the ISF/SF in the sows' diets.

Check the estrus status of sows every morning and afternoon, and then artificially inseminate them with standard doses of Duroc semen after estrus. Starting from the first artificial insemination of sows, the experimental diet was fed once a day at 8:30 am and 14:30 pm, with a total of 2.3 kg of feed per day. The ambient temperature is controlled at 16–20 °C and the humidity is 60–70%. Sows were slaughtered on the morning of the 19th day of gestation.

2.2. Sample collection

The sow UF was collected by washing both uterine horns with 300 mL sterile Phosphate Buffered Saline (PBS), and then centrifugation at 5000 ×*g* for 5 min to remove cells and cell debris. Divided into 50 mL centrifuge tubes and stored at −80 °C to be measured.

2.3. Determination of reproductive hormones and inflammatory factors

The concentrations of progesterone (PROG), estradiol (E_2), tumor necrosis factorα (TNF-α) and interleukin-6 (IL-6) in UF were determined by enzyme-linked immunosorbent assay (ELISA) kit (Ruixin Biotechnology Co., Ltd., Quanzhou, China). All indexes were measured according to the instructions.

2.4. Identification and quantification of proteins through 4D-Label free

2.4.1. Proteins extraction and digestion

Proteins were lysed and extracted using SDT Lysis Buffer (4% SDS, 100 mM Tris HCl, 1 mM DTT, pH = 7.6), with 3 replicates selected from each group ($n = 3$). The protein concentration was determined with the BCA protein assay kit (Thermo Fisher, A53226). And the protein is digested using trypsin according to the Filter-aided sample preparation (FASP) procedure [19]. Take 300 μg of protein and add 30 μL SDT buffer, in which detergent, DTT, and other low molecular weight components are removed using UA buffer (8 M Urea, 150 mM Tris-HCl, pH = 8.0) through repeated ultrafiltration. Then add 100 μL iodoacetamide blocks the reduction of cysteine residues and incubates in the dark for 30 min and uses 100 μL wash with UA buffer 2–3 times, then use 100 μL wash twice with 25 mM NH_4HCO_3 buffer. Finally, the protein suspension was treated with 4 μg trypsin (Promega Biotech Co., Ltd., Beijing) was digested overnight in NH_4HCO_3 buffer at 37 °C, and the resulting peptides were collected as a filtrate. The collected peptides were desalinated on C18 Cartridges (Empore™ SPE Cartridges C18), followed by vacuum centrifugation and reconstituted in 40 μL 0.1% (*v*/v) formic acid. The peptide content was estimated by using a UV light at 280 nm using an extinctions coefficient of 1.1 of 0.1% (g/l) solution that was calculated on the basis of the frequency of tryptophan and tyrosine in vertebrate proteins.

2.4.2. Liquid chromatography–mass spectrometry (LC-MS/MS) analysis

LC-MS/MS analysis was performed on a timsTOF Pro mass spectrometer. The peptides were loaded onto a reverse phase trap column (Thermo Scientific Acclaim PepMap100, 100 μm * 2 cm, nanoViper C18) connected to the C18-reversed phase analytical column (Thermo Scientific Easy Column, 10 cm long, 75 μm inner diameter, 3 μm resin) in buffer A (0.1% Formic acid) and separated with a linear gradient of buffer B (84% acetonitrile and 0.1% Formic acid) at a flow rate of 300 nL/min controlled by IntelliFlow technology. The detection method of the mass spectrometer is positive ions, the ionization source voltage is set to 1.5 kV, and the scanning range of the mass spectrometer is set to 100-1700 *m/z*. The data collection mode adopts the Parallel Accumulated Serial Fragmentation (PASEF) mode, and performed 10 cycles of PASEF MS/MS with a target intensity of 1.5 k and a threshold of 2500. Active exclusion was enabled with a release time of 0.4 min. MaxQuant software (v 1.6.14) identifies and quantitatively analyzes the raw data of mass spectrometry. The relevant experimental parameters are listed in Table 1.

2.4.3. Bioinformatics analysis

Perform bioinformatics analysis on differentially expressed proteins,

Table 1
Related parameters, database, and methods in LC-MS/MS analysis.

Item	Value
Enzyme	Trypsin
Max missed cleavages	2
Main search	6 ppm
First search	20 ppm
MS/MS Tolerance	20 ppm
Fixed modifications	Carbamidomethyl (C)
Variable modifications	Oxidation (M)
Database	https://www.uniprot.org/ uniprot_Sus_scrofa_335027.fasta accessed on 14 June 2023
Database pattern	Reverse
Include contaminants	True
Protein FDR	≤0.01
Peptide FDR	≤0.01
Protein Quantification	LFQ

including cluster analysis, subcellular localization analysis, GO, KEGG annotation, and enrichment analysis. The relevant software, methods, and databases are listed in Table 2.

2.5. Western blot analysis

The uterine environment in early pregnancy is an inflammatory environment, and NF-κB, RIP2, Caspase 8 and ITPR3 are related to inflammatory responses. The proteomic results showed that the abundance of RIP2, Caspase 8 and ITPR3 in the TRE was significantly lower than that in the CON ($P < 0.05$). Therefore, western blot was used to determine the expression of NF-κB, RIP2, Caspase 8 and ITPR3 proteins in UF to verify the accuracy of the 4D-lable free results. Firstly, total protein was extracted from UF using RIPA working solution (Beyotime, P0013D, contains protease inhibitors), and then the total protein concentration was determined using the BCA kit (Thermo Fisher, A53226). Add 5 × loading buffer (Beyotime, P0015L) to the total protein solution of each sample, vortex mix well, and heat at 98 °C for 10 min. Use SDS-PAGE gel quick preparation kit (Beyotime, P0012AC) to prepare 10% separation gel and 5% concentration gel. The voltage of the concentrated gel is 80 V, and the voltage of the separated gel is 130 V. The electrophoresis ends when the strip reaches the bottom of the glass plate. Then transfer the gel to the polyvinylidene fluoride membrane (PVDF) membrane (Millipore, IPVH00010). After the transfer is completed, use 1 × TBST wash the membrane for 5 min and then closed with 1% BSA for 1 h. The membranes were incubated with primary antibodies NF-κB (Servicebio, GB11997, 1:1000), RIP2 (Huabio, ER1915–87, 1:1000), Caspase 8 (Proteintech, 66093–1-IG, 1:1000) and ITPR3 (ABclonal, A23202, 1:1000), incubated overnight at 4 °C. Then use 1 × TBST washing membrane three times and closed with 5% skimmed milk powder containing secondary antibody for 1 h. After washing, use an ultrasensitive ECL chemiluminescence reagent kit (Beyotime, P0018S) for color reaction and use Image J (v 1.58) software to analyze the grayscale values of protein bands.

2.6. Statistical analysis

The data of reproductive hormones, inflammatory factors and protein expression were subjected to independent sample *t*-test using IBM SPSS Statistics 27 software. The results are expressed as "mean ± SE", with $P < 0.05$ indicating a significant difference and $0.05 \leq P < 0.1$ indicating a significant trend. The proteomics raw data have been deposited to the ProteomeXchange Consortium (https://proteomecentral.proteomexchange.org), the project name is 4D-Lable free proteomics of sow uterine fluid and dataset identifier PXD048747. In the screening of significantly different expressed proteins, the number of up-regulated and down-regulated proteins between the comparison groups was obtained by using a Fold Change (FC) >1.5 (up regulation>1.5 times or down regulation<0.67 times) and *P*-value <0.05 (*t*-test).

Table 2
Related methods, databases, and software in bioinformatics analysis.

Analysis	Database/software/methods
Cluster analysis	Cluster 3.0 (http://bonsai.hgc.jp/~mdehoon/software/cluster/software.htm) and Java Treeview software (http://jtreeview.sourceforge.net)
Domain annotation	Pfam database and InterProScan software
GO annotation	Blast2GO https://www.blast2go.com/
KEGG annotation	KEGG database http://geneontology.org/
Enrichment analysis	Fisher's Exact Test

3. Results

3.1. ISF/SF changed the reproductive hormone and inflammatory factor concentration in sow UF

Compared with the CON, the concentration of PROG in UF of the TRE was significantly increased ($P<0.01$, Fig. 1B), and the concentration of TNF-α in UF of the TRE was significantly decreased($P<0.05$, Fig. 1C), while there was no significant difference in E_2 and IL-6 concentration between the two groups ($P>0.05$, Fig. 1A and D).

3.2. Identification of differential abundance proteins (DAPs)

Through the 4D-Label free quantitative protein detection technology, we obtained a total of 24,713 unique peptides and 4248 proteins in the CON and TRE (Supplementary Table S1). The overlap of identified proteins between CON and TRE was analyzed using Venn diagrams (Fig. 2A). The CON group and the TRE group together contained 4060 proteins. The CON group were specific to the 134 proteins and the TRE group were specific to the 53 proteins. The abundance Fold Change (up-regulated>1.5 or down-regulated<0.67) and $P < 0.05$ (*t*-test) were used as criteria to obtain the number of DAPs in the CON and TRE. Compared with the CON, the TRE had 38 proteins abundance upregulated and 283 proteins abundance downregulated (Fig. 2B and C, Supplementary Table S2).

3.3. Cluster analysis of DAPs

Hierarchical Cluster was used to perform cluster analysis on DAPs between the control group and the treatment group, and presented in the form of a heatmap (Fig. 3). The DAPs obtained through screening with FC > 1.5 and $P < 0.05$ (t-test) can obviously separate the CON and TRE, indicating that DAPs screening can represent the impact of experimental treatment on the on the sample.

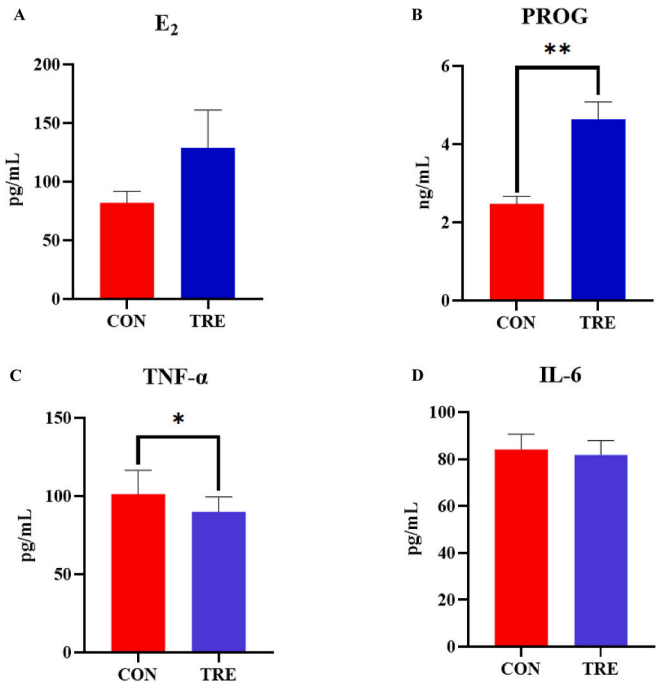

Fig. 1. The concentration of E2, PROG, TNF-α and IL-6 in UF. E2: estradiol, PROG: progesterone, TNF-α: Tumor necrosis factor α, IL-6: Interleukin-6. Data are means ± standard errors. $n = 8$, $^{*}P < 0.05$, $^{**}P < 0.01$. CON: sows fed basal diet; TRE: sows fed basal diet +11 g/kg Inulin.

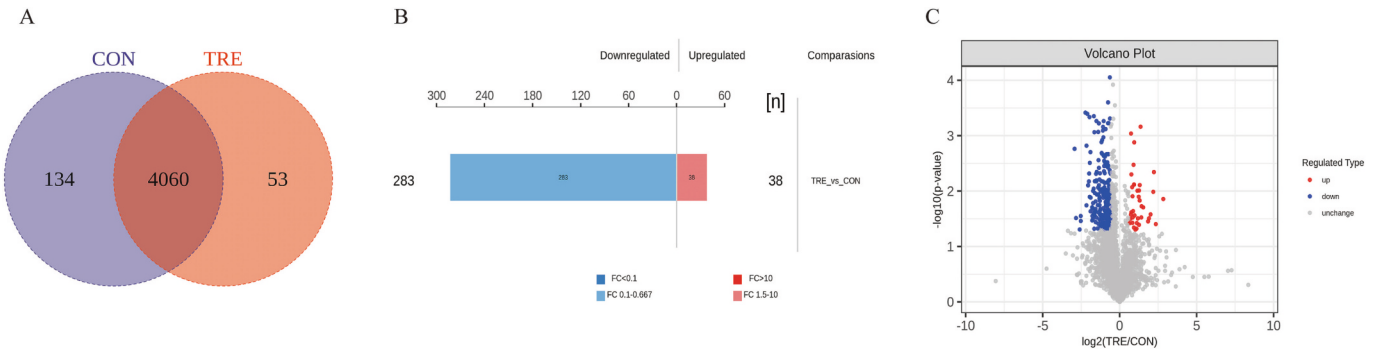

Fig. 2. Overall identification of protein abundance and identification and analysis of differentially abundant proteins. (A) Distribution of proteins in the CON and TRE. (B) and (C)The number of proteins with up and down-regulated abundance levels in the CON and TRE. CON: sows fed basal diet; TRE: sows fed basal diet +11 g/kg Inulin.

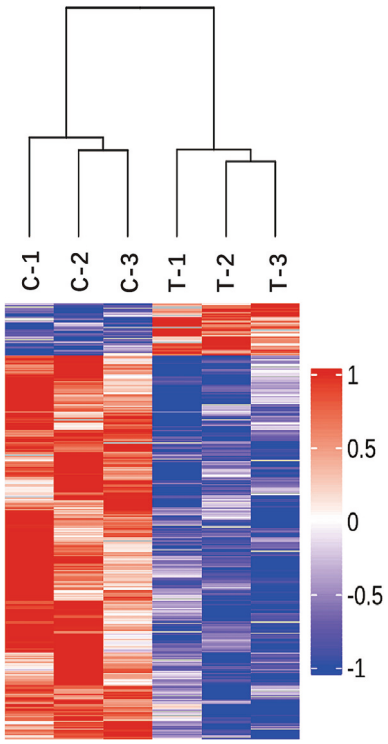

Fig. 3. Cluster analysis of significantly differentially expressed proteins between TRE group and CON group. Each column represents a group of samples, each row represents a protein, and the expression of DAPs in different samples is standardized using the z-score method and displayed in the heat map without color. Red represents up-regulated protein expression, blue represents down-regulated protein expression. C: sows fed basal diet; T: sows fed basal diet +11 g/kg Inulin. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.4. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis of DAPs

GO functional annotation used to enrich the DAPs from three aspects: Biological Process (BP), Cellular Component (CC) and Molecular Function (MF). In BP, we found that DAPs are mainly enriched in vesicle-mediated transport, vesicle docking involved in exocytosis, vesicle docking, cytoplasmic vesicle part and inorganic cation transmembrane transporter activity (Fig. 4A, Supplementary Table S3). In CC, DAPs are mainly related to the tethering complex, endosomal part, Golgi apparatus part, and cytoplasmic vesicle part (Fig. 4B, Supplementary Table S3). The MF of DAPs is mainly related to transfer activity, transfer phosphorus-containing groups, kinase activity, protein kinase activity, ATP binding and cation transmembrane transporter activity (Fig. 4C, Supplementary Table S3). The enrichment analysis of KEGG pathway showed that the DAPs are mainly enriched NOD-like receptor signaling pathway, Insulin resistance and Glutamatergic synapse (Fig. 5, Supplementary Table S4).

3.5. Validation of inflammatory related proteins in UF DAPs

We selected three inflammation-related proteins from the DAPs to validate the proteomics results. The results of western blot showed that the expression of RIP2, ITPR3 and Caspase8 in UF of the TRE decreased significantly compared to the CON ($P < 0.01$, Fig. 6). In addition, our western blot results showed that the expression of NF-κB in TRE was significantly decreased than in the CON ($P < 0.01$, Fig. 6).

4. Discussion

UF is produced by the secretion of endometrial glands and epithelial cells, and its volume and nutrients are in a dynamic process of change [20–22]. As the pregnancy progresses, when the embryo reaches the location of implantation in the uterus, UF is quickly absorbed, resulting in the closure of the uterine cavity and the formation of an environment conducive to embryo implantation [4,23,24]. Previous studies have shown that PROG promotes the absorption of UF by the uterus during embryo implantation, thereby promoting embryo implantation [22,25]. In addition, PROG can also improve the transport of nutrients in the uterus and the composition of nutrients such as amino acids and lipids in the UF, thus regulating embryonic development [26–28]. Our results show that the concentration of PROG in the UF of TRE is significantly higher than that of CON. Previous studies have shown that dietary supplementation with soluble fiber can improve the reproductive performance of sows, reduce the coefficient of variation in the litter, and increase litter size [12,29]. Therefore, we speculated that ISF/SF = 4.89 could improve the utilization of nutrients by embryos, promote embryo implantation and improve reproductive performance by increasing the concentration of PROG in UF.

The implantation of pig embryos starts from 11 to 13 days of pregnancy and is completed around 25 days of pregnancy [30]. During this period, the shape of the embryo will become spherical and filamentous, gradually forming the shape of the fetus [31]. We identified 4248 proteins in UF using 4D-Lable Free, which is more than the protein identified by Hu et al. [14]. This may be due to He et al. performed the experiments in a different mass spec using different liquid chromatography gradient, and 4D-Lable Free can cover a larger proteome region compared to other isotope labeling methods [14,32]. In addition, the experimental sows in this study had different breeds and parity compared to the experimental sows studied by He et al., which may be

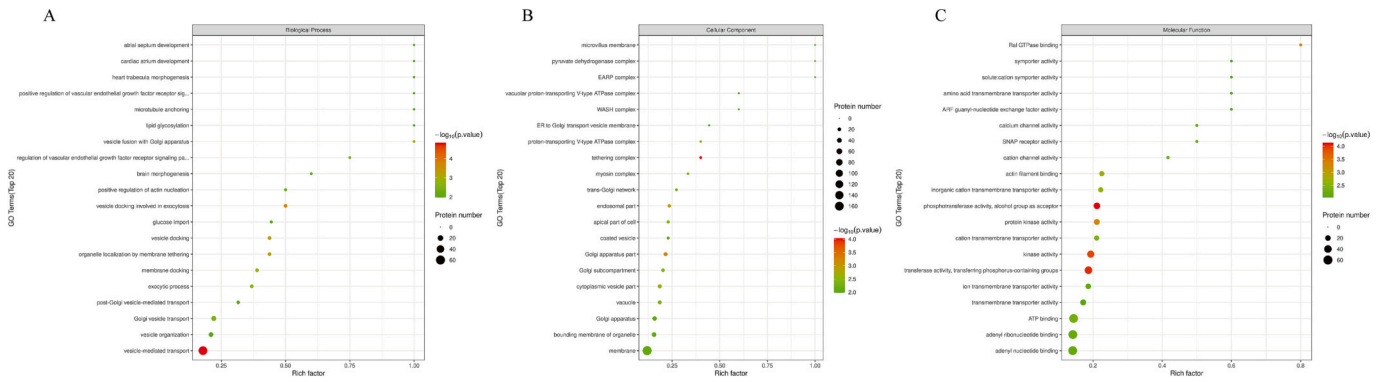

Fig. 4. GO functional annotation and enrichment analysis of DAPs (A) GO enrichment in Biological Process (Top 20). (B) GO enrichment in Cellular Component (Top 20). (C) GO enrichment in Molecular Function. Rich Factor: represents the proportion of the number of differentially expressed proteins annotated to a GO functional class to the number of all identified proteins annotated to that GO functional class. The color gradient represents the size of the *P*-value (−log10).

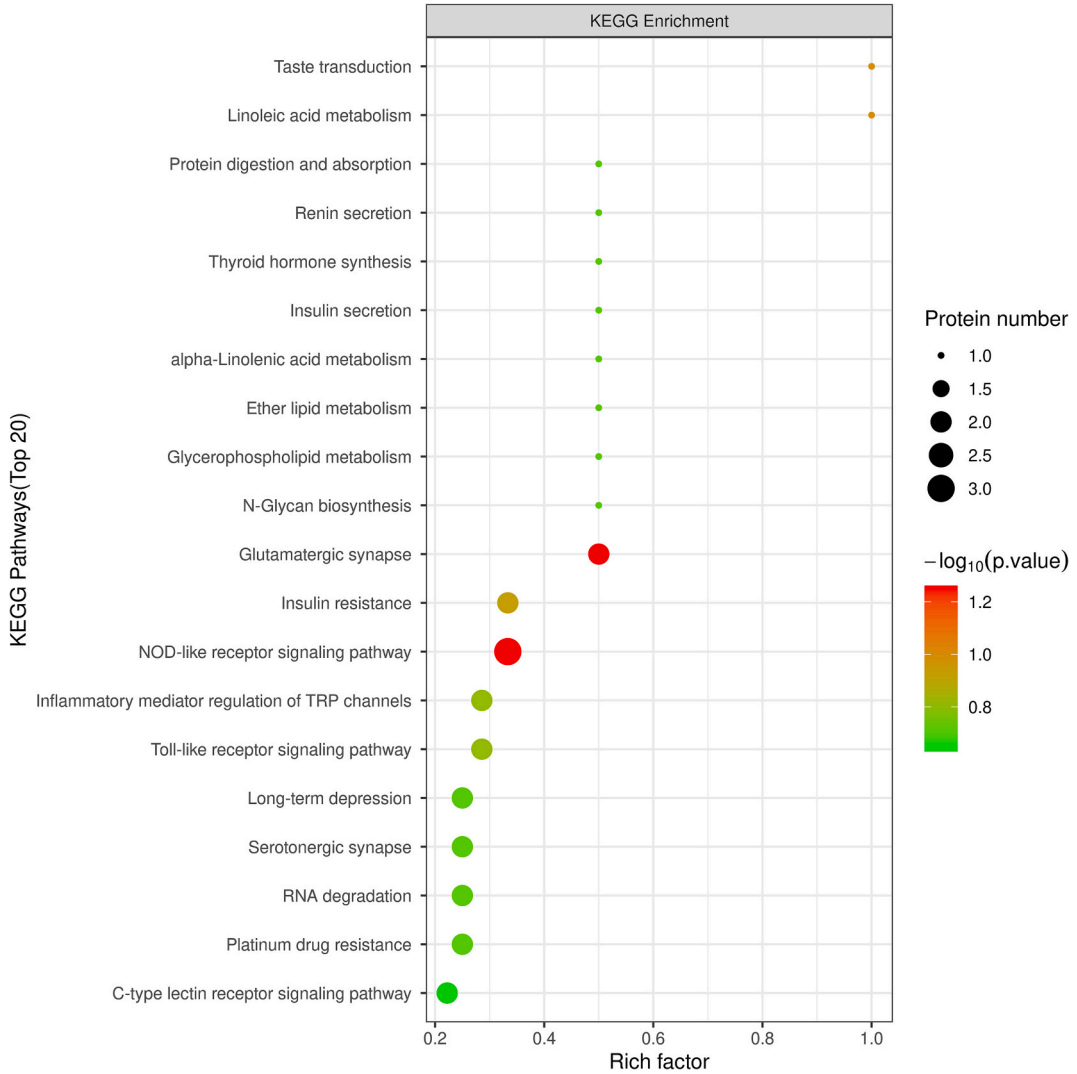

Fig. 5. KEGG pathway enrichment analysis of DAPs. Annotation and attribution of KEGG pathway for differentially expressed proteins (Top 20). Rich Factor: represents the proportion of the number of differentially expressed proteins annotated to a KEGG pathway to the number of all identified proteins annotated to that KEGG pathway. The color gradient represents the size of the *P*-value (−log10).

one of the reasons for the differences in proteomics results. Through GO annotation and enrichment analysis, we found that the biological processes involved in DAPs are mainly related to vesicle transport and docking between vesicles, and the biological functions of these proteins are mainly related to the binding of transporters, protein kinases, and ATP. Exosomes are a type of extracellular vesicles (EV) with a diameter of 30–150 nm. They contain proteins, lipids, nucleic acids, and various cytokines, and participate in information exchange between cells

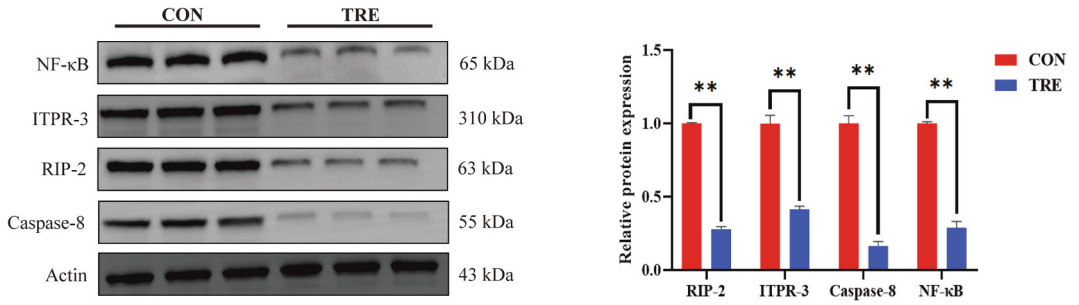

Fig. 6. Western blot analysis of differentially abundance proteins in uterine fluid. RIP2: Receptor-interacting serine/threonine-protein kinase 2; ITPR3: Inositol 1,4,5-Trisphosphate Receptor Type 3, NF-κB: Nuclear factor kappa-B. Data are means ± standard errors. $n = 3$, $^{*}P < 0.05$, $^{**}P < 0.01$. CON: sows fed basal diet; TRE: sows fed basal diet +11 g/kg Inulin.

[33,34]. Previous studies have shown that UF is rich in exosomes, which can regulate the adhesion of ectodermal cells and the migration and proliferation of trophoblast cells during the embryonic attachment period [7,35]. Studies have shown that the synthesis and secretion of exosomes are closely related to energy utilization, vesicle transport, and recognition among vesicles [33,36]. The study of KaLynn et al. also proved that most of the proteins in the uterus during the implantation period were related to EV [37]. Therefore, we conclude that fiber in the diet can regulate the signal transmission of EV in UF, thereby regulating maternal inter-embryonic information exchange and affecting embryo implantation. In this study, it is a pity that we did not isolate extracellular vesicles from UF, limiting the analysis of the effect of diet on sow embryo implantation from the perspective of extracellular vesicles. Therefore, the effect of diet on EV from UF could be further investigated in the future.

Due to the up-regulated proteins enriched pathway in KEEG analysis only contains one DAP, while the downregulated protein enriched pathway contains multiple DAPs with higher reliability. Therefore, we will only discuss the pathways of protein enrichment that are down-regulated. KEGG-enriched down-regulated DAPs are mainly involved in NOD like receptor signaling pathway, Insulin resistance, and Glutamatic synapse. As we all know, the uterus in early pregnancy is a pro-inflammatory microenvironment, which is extremely important for embryo implantation [38]. NOD-like receptor signaling pathway is usually used as an inflammatory signaling pathway, which can relies on RIP2 to activate downstream NF-κB [39] and MAPK [40] to activate a series of inflammatory reactions and the secretion of proinflammatory cytokines. Moreover, RIP2 can enhance the activity of Caspase8 and induce programmed cell death [41]. ITPR3, which is the site of calcium channel between mitochondria and endoplasmic reticulum, mediates Ca^{2+} release from endoplasmic reticulum to mitochondria [42]. Previous studies have shown that persistent inflammation up-regulates the expression of NF-κB and ITPR3 in the heart of chickens, leading to calcium dysregulation [43]. Notably, our results of western blot showed that the expression of NF-κB, RIP2, Caspase 8 and ITPR3 protein and the concentration of pro-inflammatory cytokine TNF-α in UF of TRE were significantly decreased. These results not only demonstrate the reliability of 4D-Lable free technology, but also indicate that dietary ISF/SF can reduce the expression of inflammation-related proteins and release of cytokines to regulate the inflammatory environment of uterus. Previous studies have shown that excessive inflammation in the uterus can lead to preeclampsia or embryo implantation failure [44–46]. Therefore, the sow dietary ISF/SF may regulate embryo implantation by regulating the inflammatory environment in the uterus. Down-regulated DAPs are also involved in Insulin resistance. However, Insulin resistance reduces uterine receptivity and leads to mitochondrial dysfunction leading to oxidative stress resulting in failure of embryo attachment [47,48]. This implying that dietary ISF/SF can inhibit insulin resistance in the uterus and thus regulate embryo implantation. It is a pity that we have not found a connection between Glutamatic synapse and embryo implantation through extensive literature review, and further research is needed to explain this.

However, present study found that there is no significant difference in embryo number, backfat thickness or body weight between CON and TRE pregnant sows on day 19 of pregnant (Supplementary Table S5). Considering that the embryo implantation of sows is completed around 25 days of pregnancy, and the slaughter time of sows in this study is on the day 19 of pregnancy, the embryo implantation has not yet been completed, which may be the reason why there is no significant difference in the number of embryos. Therefore, it may be necessary to extend the gestation time of the sow to determine the effect of dietary ISF/SF on embryo number. In addition, studies have shown that dietary fiber supplementation has no effect on the reproductive performance of sows [49,50], which may be due to different types of fiber supplementation or different ISF/SF of the diet, this may require further study. Notably, our previous research has shown that appropriate dietary fiber levels can improve animal reproductive performance [11,51,52], other studies have also demonstrated the important role of fiber in improving sow reproductive performance [53]. Therefore, we can conclude that dietary fiber composition affects early embryo implantation by regulating specific protein abundances in the UF of sows during early pregnancy.

5. Conclusion

In conclusion, this study revealed the effect of fiber in sow diet on UF from a proteomic perspective using 4D-Label free technology. The results showed that ISF/SF = 4.96 in the diet of sows increase the concentration of PROG in UF, reduce the concentration of pro-inflammatory cytokines TNF-α and the abundance of inflammatory and insulin resistance related proteins in UF, alleviate the inflammatory environment in the uterus, and thus regulate early embryo implantation. In addition, the research results also provide theoretical references for future research on improving sow reproductive performance, as well as early miscarriage and reproductive nutrition in human pregnancy.

Author contributions

The authors' responsibilities were as follows: Shengyu Xu conceptualized this project and obtained funding. Junlei Chang and Xujing Pan carried out the animal experiments and performed the laboratory work. Junjie Gao, Yong Zhuo, Xuemei Jiang, Lianqiang Che, Yan Lin, Zhengfeng Fang, Bin Feng, Jian Li, Lun Hua, Xilun Zhao, Ruinan Zhang and De Wu provided the resources and performed the statistical analysis. Junlei Chang wrote the paper. All authors critically reviewed the manuscript and gave final approval for the version to be published.

CRediT authorship contribution statement

Junlei Chang: Writing – original draft, Data curation. **Xujing Pan:** Formal analysis. **Junjie Gao:** Software, Resources. **Yong Zhuo:**

Software, Resources. **Xuemei Jiang:** Software, Resources. **Lianqiang Che:** Software, Resources. **Yan Lin:** Software, Resources. **Zhengfeng Fang:** Software, Resources. **Bin Feng:** Software, Resources. **Jian Li:** Software, Resources. **Lun Hua:** Software, Resources. **Xilun Zhao:** Software, Resources. **Ruinan Zhang:** Software, Resources. **De Wu:** Software, Resources. **Shengyu Xu:** Writing – review & editing, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare no conflicts of interest associated with this study.

Data availability

I have shared the link to my data at the Supplementary data step.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jprot.2024.105123.

References

[1] M.M. Swindle, A. Makin, A.J. Herron, F.J. Clubb Jr., K.S. Frazier, Swine as models in biomedical research and toxicology testing, Vet. Pathol. 49 (2) (2012) 344–356, https://doi.org/10.1177/0300985811402846.
[2] K. Gutierrez, N. Dicks, W.G. Glanzner, L.B. Agellon, V. Bordignon, Efficacy of the porcine species in biomedical research, Front. Genet. 6 (2015) 293, https://doi.org/10.3389/fgene.2015.00293.
[3] R.T. Kridli, K. Khalaj, M. Bidarimath, C. Tayade, Placentation, maternal-fetal interface, and conceptus loss in swine, Theriogenology. 85 (1) (2016) 135–144, https://doi.org/10.1016/j.theriogenology.2015.08.001.
[4] Y. Zhang, Q. Wang, H. Wang, E. Duan, Uterine fluid in pregnancy: a biological and clinical outlook, Trends Mol. Med. 23 (7) (2017) 604–614, https://doi.org/10.1016/j.molmed.2017.05.002.
[5] P.T. Ruane, T. Garner, L. Parsons, P.A. Babbington, I. Wangsaputra, S.J. Kimber, A. Stevens, M. Westwood, D.R. Brison, J.D. Aplin, Trophectoderm differentiation to invasive syncytiotrophoblast is promoted by endometrial epithelial cells during human embryo implantation, Hum. Reprod. 37 (4) (2022) 777–792, https://doi.org/10.1093/humrep/deac008.
[6] M. Gauster, G. Moser, S. Wernitznig, N. Kupper, B. Huppertz, Early human trophoblast development: from morphology to function, Cell. Mol. Life Sci. 79 (6) (2022) 345, https://doi.org/10.1007/s00018-022-04377-0.
[7] Q. Hu, X. Zang, Y. Ding, T. Gu, J. Shi, Z. Li, G. Cai, D. Liu, Z. Wu, L. Hong, Porcine uterine luminal fluid-derived extracellular vesicles improve conceptus-endometrial interaction during implantation, Theriogenology. 178 (2022) 8–17, https://doi.org/10.1016/j.theriogenology.2021.10.021.
[8] H. Bhatt, L.J. Brunet, C.L. Stewart, Uterine expression of leukemia inhibitory factor coincides with the onset of blastocyst implantation, Proc. Natl. Acad. Sci. U. S. A. 88 (24) (1991) 11408–11412, https://doi.org/10.1073/pnas.88.24.11408.
[9] C.A. White, J.G. Zhang, L.A. Salamonsen, M. Baca, W.D. Fairlie, D. Metcalf, N. A. Nicola, L. Robb, E. Dimitriadis, Blocking LIF action in the uterus by using a PEGylated antagonist prevents implantation: a nonhormonal contraceptive strategy, Proc. Natl. Acad. Sci. U. S. A. 104 (49) (2007) 19357–19362, https://doi.org/10.1073/pnas.0710110104.
[10] Y.C. Ruan, J.H. Guo, X. Liu, R. Zhang, L.L. Tsang, J.D. Dong, H. Chen, M.K. Yu, X. Jiang, X.H. Zhang, K.L. Fok, Y.W. Chung, H. Huang, W.L. Zhou, H.C. Chan, Activation of the epithelial Na+ channel triggers prostaglandin E_2 release and production required for embryo implantation, Nat. Med. 18 (7) (2012) 1112–1117, https://doi.org/10.1038/nm.2771.
[11] P. Zhou, Y. Zhao, P. Zhang, Y. Li, T. Gui, J. Wang, C. Jin, L. Che, J. Li, Y. Lin, S. Xu, B. Feng, Z. Fang, D. Wu, Microbial mechanistic insight into the role of inulin in improving maternal health in a pregnant sow model, Front. Microbiol. 8 (2017) 2242, https://doi.org/10.3389/fmicb.2017.02242.
[12] Y. Zhuo, X. Shi, G. Lv, L. Hua, P. Zhou, L. Che, Z. Fang, Y. Lin, S. Xu, J. Li, B. Feng, D. Wu, Beneficial effects of dietary soluble fiber supplementation in replacement gilts: pubertal onset and subsequent performance, Anim. Reprod. Sci. 186 (2017) 11–20, https://doi.org/10.1016/j.anireprosci.2017.08.007.
[13] Y. Li, L. Zhang, H. Liu, Y. Yang, J. He, M. Cao, M. Yang, W. Zhong, Y. Lin, Y. Zhuo, Z. Fang, L. Che, B. Feng, S. Xu, J. Li, X. Zhao, X. Jiang, D. Wu, Effects of the ratio of insoluble fiber to soluble fiber in gestation diets on sow performance and offspring intestinal development, Animals (Basel). 9 (7) (2019), https://doi.org/10.3390/ani9070422.
[14] Y. He, X. Zang, J. Kuang, H. Yang, T. Gu, J. Yang, Z. Li, E. Zheng, Z. Xu, G. Cai, Z. Wu, L. Hong, iTRAQ-based quantitative proteomic analysis of porcine uterine fluid during pre-implantation period of pregnancy, J. Proteome 261 (2022) 104570, https://doi.org/10.1016/j.jprot.2022.104570.
[15] A.J. Kermack, S. Finn-Sell, Y.C. Cheong, N. Brook, J.J. Eckert, N.S. Macklon, F. D. Houghton, Amino acid composition of human uterine fluid: association with age, lifestyle and gynaecological pathology, Hum. Reprod. 30 (4) (2015) 917–924, https://doi.org/10.1093/humrep/dev008.
[16] C. Sun, M.A. Velazquez, S. Marfy-Smith, B. Sheth, A. Cox, D.A. Johnston, N. Smyth, T.P. Fleming, Mouse early extra-embryonic lineages activate compensatory endocytosis in response to poor maternal nutrition, Development. 141 (5) (2014) 1140–1150, https://doi.org/10.1242/dev.103952.
[17] M. Shoaib, A. Shehzad, M. Omar, A. Rakha, H. Raza, H.R. Sharif, A. Shakeel, A. Ansari, S. Niazi, Inulin: properties, health benefits and food applications, Carbohydr. Polym. 147 (2016) 444–454, https://doi.org/10.1016/j.carbpol.2016.04.020.
[18] M. Bucław, The use of inulin in poultry feeding: a review, J. Anim. Physiol. Anim. Nutr. (Berl). 100 (6) (2016) 1015–1022, https://doi.org/10.1111/jpn.12484.
[19] J.R. Wiśniewski, A. Zougman, N. Nagaraj, M. Mann, Universal sample preparation method for proteome analysis, Nat. Methods 6 (5) (2009) 359–362, https://doi.org/10.1038/nmeth.1322.
[20] C.A. Gray, R.C. Burghardt, G.A. Johnson, F.W. Bazer, T.E. Spencer, Evidence that absence of endometrial gland secretions in uterine gland knockout ewes compromises conceptus survival and elongation, Reproduction. 124 (2) (2002) 289–300.
[21] K.M. Halloran, E.C. Hoskins, C. Stenhouse, R.M. Moses, K.A. Dunlap, M. C. Satterfield, H. Seo, G.A. Johnson, G. Wu, F.W. Bazer, Pre-implantation exogenous progesterone and pregnancy in sheep. II. Effects on fetal-placental development and nutrient transporters in late pregnancy, J. Anim. Sci. Biotechnol. 12 (1) (2021) 46, https://doi.org/10.1186/s40104-021-00567-1.
[22] Y. Li, T.E. Martin, J.M. Hancock, R. Li, S. Viswanathan, J.P. Lydon, Y. Zheng, X. Ye, Visualization of preimplantation uterine fluid absorption in mice using Alexa Fluor™ 488 Hydrazide, Biol. Reprod. 108 (2) (2023) 204–217, https://doi.org/10.1093/biolre/ioac198.
[23] C.A. Clemetson, U.L. Verma, S.J. De Carlo, Secretion and reabsorption of uterine luminal fluid in rats, J. Reprod. Fertil. 49 (2) (1977) 183–187, https://doi.org/10.1530/jrf.0.0490183.
[24] X. Ye, Uterine luminal epithelium as the transient gateway for embryo implantation, Trends Endocrinol. Metab. 31 (2) (2020) 165–180, https://doi.org/10.1016/j.tem.2019.11.008.
[25] Y. Zhang, Q. Chen, H. Zhang, Q. Wang, R. Li, Y. Jin, H. Wang, T. Ma, J. Qiao, E. Duan, Aquaporin-dependent excessive intrauterine fluid accumulation is a major contributor in hyper-estrogen induced aberrant embryo implantation, Cell Res. 25 (1) (2015) 139–142, https://doi.org/10.1038/cr.2014.139.
[26] M.C. Satterfield, F.W. Bazer, T.E. Spencer, Progesterone regulation of preimplantation conceptus growth and galectin 15 (LGALS15) in the ovine uterus, Biol. Reprod. 75 (2) (2006) 289–296, https://doi.org/10.1095/biolreprod.106.052944.
[27] C.A. Simintiras, J.M. Sánchez, M. McDonald, P. Lonergan, Progesterone alters the bovine uterine fluid lipidome during the period of elongation, Reproduction. 157 (4) (2019) 399–411, https://doi.org/10.1530/rep-18-0615.
[28] C.A. Simintiras, J.M. Sánchez, M. McDonald, T. Martins, M. Binelli, P. Lonergan, Biochemical characterization of progesterone-induced alterations in bovine uterine fluid amino acid and carbohydrate composition during the conceptus elongation window, Biol. Reprod. 100 (3) (2019) 672–685, https://doi.org/10.1093/biolre/ioy234.
[29] Y. Hu, X. Huang, X. Zong, Z. Bi, Y. Cheng, X. Xiao, F. Wang, Y. Wang, Z. Lu, Chicory fibre improves reproductive performance of pregnant rats involving in altering intestinal microbiota composition, J. Appl. Microbiol. 129 (6) (2020) 1693–1705, https://doi.org/10.1111/jam.14679.
[30] G.A. Johnson, F.W. Bazer, H. Seo, The early stages of implantation and placentation in the pig, Adv. Anat. Embryol. Cell Biol. 234 (2021) 61–89, https://doi.org/10.1007/978-3-030-77360-1_5.
[31] L. Llobat, Embryo gene expression in pig pregnancy, Reprod. Domest. Anim. 55 (4) (2020) 523–529, https://doi.org/10.1111/rda.13647.
[32] M. Bantscheff, M. Schirle, G. Sweetman, J. Rick, B. Kuster, Quantitative mass spectrometry in proteomics: a critical review, Anal. Bioanal. Chem. 389 (4) (2007) 1017–1031, https://doi.org/10.1007/s00216-007-1486-6.
[33] R. Kalluri, V.S. LeBleu, The biology, function, and biomedical applications of exosomes, Science. 367 (6478) (2020), https://doi.org/10.1126/science.aau6977.
[34] L. Milane, A. Singh, G. Mattheolabakis, M. Suresh, M.M. Amiji, Exosome mediated communication within the tumor microenvironment, J. Control. Release 219 (2015) 278–294, https://doi.org/10.1016/j.jconrel.2015.06.029.
[35] D.W. Greening, H.P. Nguyen, K. Elgass, R.J. Simpson, L.A. Salamonsen, Human endometrial exosomes contain hormone-specific cargo modulating trophoblast adhesive capacity: insights into endometrial-embryo interactions, Biol. Reprod. 94 (2) (2016) 38, https://doi.org/10.1095/biolreprod.115.134890.

[36] M. Mathieu, L. Martin-Jaular, G. Lavieu, C. Théry, Specificities of secretion and uptake of exosomes and other extracellular vesicles for cell-to-cell communication, Nat. Cell Biol. 21 (1) (2019) 9–17, https://doi.org/10.1038/s41556-018-0250-9.
[37] K. Harlow, E. Taylor, T. Casey, V. Hedrick, T. Sobreira, U.K. Aryal, R.P. Lemenager, B. Funnell, K. Stewart, Diet impacts pre-implantation histotroph proteomes in beef cattle, J. Proteome Res. 17 (6) (2018) 2144–2155, https://doi.org/10.1021/acs.jproteome.8b00077.
[38] S.A. Robertson, A.S. Care, L.M. Moldenhauer, Regulatory T cells in embryo implantation and the immune response to pregnancy, J. Clin. Invest. 128 (10) (2018) 4224–4235, https://doi.org/10.1172/jci122182.
[39] P. Hysi, M. Kabesch, M.F. Moffatt, M. Schedel, D. Carr, Y. Zhang, B. Boardman, E. von Mutius, S.K. Weiland, W. Leupold, C. Fritzsch, N. Klopp, A.W. Musk, A. James, G. Nunez, N. Inohara, W.O. Cookson, NOD1 variation, immunoglobulin E and asthma, Hum. Mol. Genet. 14 (7) (2005) 935–941, https://doi.org/10.1093/hmg/ddi087.
[40] N. Inohara, T. Koseki, J. Lin, L. del Peso, P.C. Lucas, F.F. Chen, Y. Ogura, G. Núñez, An induced proximity model for NF-kappa B activation in the Nod1/RICK and RIP signaling pathways, J. Biol. Chem. 275 (36) (2000) 27823–27831, https://doi.org/10.1074/jbc.M003415200.
[41] A. Krieg, G. Le Negrate, J.C. Reed, RIP2-beta: a novel alternative mRNA splice variant of the receptor interacting protein kinase RIP2, Mol. Immunol. 46 (6) (2009) 1163–1170, https://doi.org/10.1016/j.molimm.2008.11.002.
[42] A. Mangla, M.T. Guerra, M.H. Nathanson, Type 3 inositol 1,4,5-trisphosphate receptor: a calcium channel for all seasons, Cell Calcium 85 (2020) 102132, https://doi.org/10.1016/j.ceca.2019.102132.
[43] S. Li, Y. Wang, H. Zhao, Y. He, J. Li, G. Jiang, M. Xing, NF-κB-mediated inflammation correlates with calcium overload under arsenic trioxide-induced myocardial damage in *Gallus gallus*, Chemosphere. 185 (2017) 618–627, https://doi.org/10.1016/j.chemosphere.2017.07.055.
[44] J. Kwak-Kim, S. Bao, S.K. Lee, J.W. Kim, A. Gilman-Sachs, Immunological modes of pregnancy loss: inflammation, immune effectors, and stress, Am. J. Reprod. Immunol. 72 (2) (2014) 129–140, https://doi.org/10.1111/aji.12234.
[45] C.J. Lockwood, S.J. Huang, G. Krikun, R. Caze, M. Rahman, L.F. Buchwalder, F. Schatz, Decidual hemostasis, inflammation, and angiogenesis in pre-eclampsia, Semin. Thromb. Hemost. 37 (2) (2011) 158–164, https://doi.org/10.1055/s-0030-1270344.
[46] A.S. Bhurke, I.C. Bagchi, M.K. Bagchi, Progesterone-regulated endometrial factors controlling implantation, Am. J. Reprod. Immunol. 75 (3) (2016) 237–245, https://doi.org/10.1111/aji.12473.
[47] E.M. Chang, J.E. Han, H.H. Seok, D.R. Lee, T.K. Yoon, W.S. Lee, Insulin resistance does not affect early embryo development but lowers implantation rate in in vitro maturation-in vitro fertilization-embryo transfer cycle, Clin. Endocrinol. 79 (1) (2013) 93–99, https://doi.org/10.1111/cen.12099.
[48] M. Chen, J. Li, B. Zhang, X. Zeng, X. Zeng, S. Cai, Q. Ye, G. Yang, C. Ye, L. Shang, S. Qiao, Uterine insulin sensitivity defects induced embryo implantation loss associated with mitochondrial dysfunction-triggered oxidative stress, Oxidative Med. Cell. Longev. 2021 (2021) 6655685, https://doi.org/10.1155/2021/6655685.
[49] J.A. Renteria-Flores, L.J. Johnston, G.C. Shurson, R.L. Moser, S.K. Webel, Effect of soluble and insoluble dietary fiber on embryo survival and sow performance, J. Anim. Sci. 86 (10) (2008) 2576–2584, https://doi.org/10.2527/jas.2007-0376.
[50] H.Q. Sun, Y.F. Zhou, C.Q. Tan, L.F. Zheng, J. Peng, S.W. Jiang, Effects of konjac flour inclusion in gestation diets on the nutrient digestibility, lactation feed intake and reproductive performance of sows, Animal. 8 (7) (2014) 1089–1094, https://doi.org/10.1017/S175173111400113X.
[51] L. Che, D. Feng, D. Wu, Z. Fang, Y. Lin, T. Yan, Effect of dietary fibre on reproductive performance of sows during the first two parities, Reprod. Domest. Anim. 46 (6) (2011) 1061–1066, https://doi.org/10.1111/j.1439-0531.2011.01787.x.
[52] Y. Li, H. Liu, L. Zhang, Y. Yang, Y. Lin, Y. Zhuo, Z. Fang, L. Che, B. Feng, S. Xu, J. Li, D. Wu, Maternal dietary fiber composition during gestation induces changes in offspring antioxidative capacity, inflammatory response, and gut microbiota in a sow model, Int. J. Mol. Sci. 21 (1) (2019), https://doi.org/10.3390/ijms21010031.
[53] H. Li, Z. Liu, H. Lyu, X. Gu, Z. Song, X. He, Z. Fan, Effects of dietary inulin during late gestation on sow physiology, farrowing duration and piglet performance, Anim. Reprod. Sci. 219 (2020) 106531, https://doi.org/10.1016/j.anireprosci.2020.106531.