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# Effects of maternal early gestational weight gain on skeletal muscle gene expression profile in F1 and F2 beef cattle offspring

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**Introduction:** Maternal nutrition during early gestation is associated with metabolic and immunological adaptations that may influence postnatal growth, with possible long-term and multigenerational effects.

**Methods:** This study evaluated how the rate of maternal body weight gain in F0 dams during early gestation affects gene expression in the skeletal muscle of F1 offspring and their F2 fetuses. A subset of 16 crossbred Angus heifers (F0) were assigned to a low gain (LG; 0.28 kg/day) or moderate gain (MG; 0.79 kg/day) treatment during the first 84 days of gestation.

**Results and discussion:** F1 heifers from the LG group showed upregulation of *PCK2*, *ARG2*, *PHGDH*, *PSAT1*, *PSPH*, and *ASNS*, associated with the biological processes cytochrome complex assembly, mitochondrion organization, mitochondrial gene expression, and mitochondrial transport, relative to the MG group, suggesting greater metabolic plasticity and possible compensatory adjustments related to satellite cell function and postnatal muscle hypertrophy. Conversely, the upregulation of *TNFAIP3*, *ITGB2*, *EOGT*, *BOLA-NC1*, and *C4A* in F1 heifers from the MG group, associated with the pathways cell adhesion molecules (CAMs), antigen processing and presentation, and cytokine–cytokine receptor interaction, may be related to the activation of local immunomodulatory mechanisms and to the preservation of skeletal muscle structural integrity. At the multigenerational level, the modulation of *ITGB2*, *BOLA-NC1*, *C4A*, and *TNFAIP3* in F1 and F2 progeny from the MG group, associated with enrichment of the CAMs pathway and consistent negative regulation of thermogenesis, oxidative phosphorylation, and ribosome pathways relative to the LG group, together with the positive regulation of *bta-miR-133a-1* in the LG progeny, points to the involvement of epigenetic

mechanisms in the coordination of persistent metabolic and myogenic adjustments across generations. Moreover, the maternal rate of body weight gain during early gestation is associated with metabolic changes that support postnatal hypertrophic growth and with local immunological modulation in skeletal muscle, with multigenerational molecular outcomes.

#### KEYWORDS

calves, fetal programming, maternal nutrition, multigenerational effects, RNA sequencing

## 1 Introduction

Maternal nutrition plays a crucial role in fetal development. Previous studies show that both restrictive and excessive diets during pregnancy in dams can have significant impacts on the offspring (Wallace et al., 2012; Duarte et al., 2014; Gionbelli et al., 2018; Rodrigues et al., 2020; Costa et al., 2021; Shokrollahi et al., 2024a, 2024b). The skeletal muscle has lower priority in nutrient partitioning compared to vital organs, such as the brain, liver, and heart, during fetal life (Zhu et al., 2006). Myogenesis in cattle begins in the early months of fetal life, with the peak of primary fiber formation occurring around two months of gestation (Du et al., 2010). Although the first third of gestation is not a period of intense myofiber formation, it plays a decisive role in organizing early tissue architecture and preparing the cellular environment for later myogenesis (Picard and Gagaoua, 2020). During this window, primary fibers provide the framework for secondary fibers, which ultimately make up most skeletal muscle and contribute to postnatal growth and performance (Santos et al., 2022). Because this early architecture is highly responsive to intrauterine conditions, skeletal muscle has become a key tissue for studies of developmental programming (Du et al., 2010; Reynolds et al., 2019). In this context, the *Longissimus thoracis* is widely used to examine the molecular processes that shape myogenesis and influence important traits in beef cattle production (Marquez et al., 2017; Gionbelli et al., 2018; Rodrigues et al., 2020; Costa et al., 2021; Carvalho et al., 2022; Ramírez-Zamudio et al., 2022; Nascimento et al., 2024).

In our study, during the first 84 days of gestation, we applied a nutritional plan to the F0 dams to promote two different rates of body weight gain (0.28 kg/day and 0.79 kg/day), a period that encompasses the transition from the peak of primary myogenesis to the beginning of secondary myogenesis (Du et al., 2010). While most fetal growth occurs during late gestation, the intense formation of muscle fibers by hyperplasia between the second- and sixth-months post-conception establishes the foundation for development during postnatal life (Bonnet et al., 2010; Du et al., 2010; Santos et al., 2022). Therefore, research on maternal nutrition has focused on the mid-to-late gestation periods (Moriel et al., 2021; Barcelos et al., 2022), underlining the notion that nutrition for dams in early gestation has negligible effects on fetal myogenesis (Du

et al., 2010). Although maternal nutrition during the initial stages of gestation is crucial, the care for replacement heifers during this interval is widely variable with many beef cattle producers providing supplement only in the late stages of gestation, aiming to enhance the body condition before calving (Martin et al., 2007; Bohnert et al., 2013).

We have demonstrated that maternal nutrition and rate of body weight gain during early gestation modify the expression of genes related to lipid metabolism and nutrient transport in the placenta (Diniz et al., 2021), which may influence the availability of essential substrates, such as glucose (Hay, 2006), fructose (Caton et al., 2020), and amino acids (Menezes et al., 2021), necessary for fetal growth. Regarding myogenesis at this gestational stage, it has been reported in cattle that early modifications in the embryonic development environment can impact fetal growth (Crouse et al., 2019; Menezes et al., 2022) and subsequently the postnatal development of the offspring (Nishino et al., 2025; Costa et al., 2021). Although some studies in ewes have shown that nutritional changes, such as a 30% restriction or a 50% excess during the periconceptional period, can alter the expression of myogenic regulatory factors and proteins involved in myofiber formation (Lie et al., 2015; Tong et al., 2009), these results arise from undernutrition or overfeeding models. Far less is known about how differences in maternal weight gain during early gestation, as those proposed in this study and more representative of beef cattle production systems (Baumgaertner et al., 2024a), affect skeletal muscle development and the gene expression patterns associated with metabolic pathways and other essential physiological functions.

Studies revealed that the influence of a direct environmental factor may not be limited to the immediately exposed generation (Skinner, 2011). This phenomenon is known as non-genetic inheritance and is understood through epigenetics, which examines changes in gene expression caused by modifications in the environment around the genes without altering the DNA sequence (Thompson et al., 2020). It is important to note that epigenetic inheritance can involve transmitting epigenetic information through the germline to subsequent generations that were not directly exposed to an environmental effect (Skinner et al., 2010). In contrast, multigenerational epigenetics explains that exposure to environmental factors during embryonic gonadal sex

determination and the germ cells of the first-generation (F1) results in direct exposure to the subsequent offspring (Skinner, 2008; Manikkam et al., 2012). There is a scarcity of studies on farm animals demonstrating the effect of maternal gestational nutrition on offspring outcomes over multiple generations (Shasa et al., 2015; Pankey et al., 2017). Evaluating the F2 generation allows us to determine whether the molecular adjustments observed in the directly exposed progeny also occur in F2 itself.

Additionally, in beef cattle, the understanding of the effects caused by a maternal nutrition model during early gestation, targeting two rates of body weight gain within the range observed in commercial breeding systems, on the gene regulation of skeletal muscle has yet to be explored. Herein we tested the hypothesis that maternal nutrition aiming at two rates of body weight gain (low [LG] or moderate [MG] gain) during early gestation would affect the gene expression of skeletal muscle in both the F1 offspring and the subsequent F2 generation. Thus, our aim was to measure the gene expression profiles and identify differentially expressed genes (DEG), metabolic pathways (KEGG), and biological processes (GO BP) in the skeletal muscle of F1 heifers at birth, as well as during the early gestation phase, applying the same approach to F2 fetuses.

## 2 Materials and methods

All experiments and procedures were carried out in strict adherence to the applicable guidelines and regulations. The experimental design, animal care, and tissue collection protocols were approved by the North Dakota State University Institutional Animal Care and Use Committee (IACUC #A19062 and #A20047).

### 2.1 Animals, experimental design, housing, and tissue collection

A subgroup of sixteen F1 heifers and their F2 fetuses were selected from a previous trial in which 100 F0 dams underwent a nutritional treatment for two rates of body weight gain in early gestation (Baumgaertner et al., 2024a). In summary, crossbred Angus heifers (F0), individually fed through the Insentec system (Hokofarm B.V., Marknesse, Flevoland, Netherlands), were synchronized and artificially inseminated (AI) with sexed semen from a single sire. The procedures were conducted at the NDSU Beef Cattle Research Complex (BCRC; Fargo, ND). The treatments were randomly assigned during the AI process, with F0 heifers designated to achieve gains of 0.28 kg/day (LG; n = 50) or 0.79 kg/day (MG; n = 50) during the first 84 days of gestation. Both groups of F0 heifers received a basal diet of 37% corn silage, 53% prairie hay, and 10% distillers dried grain with solubles (DDGS). Additionally, F0 heifers from the MG group received daily 0.58% of body weight (BW) on a dry matter (DM) basis of an energy/protein supplement composed of ground corn, DDGS, wheat midds, fish oil, urea, and ethoxyquin, incorporated into a total mixed ration (TMR) (Table 1). After day 84 of gestation, F0 heifers

TABLE 1 Feed ingredients and nutrient characteristics of the diets offered to F0 dams during the first 84 days of gestation.

Ingredient, % of DM	Treatment <sup>1</sup>	
	LG	MG
Prairie hay	53	41
Corn silage	37	29
DDGS	10	5
Energy/protein supplement <sup>2</sup>	–	25
Chemical composition		
DM, %	51.6	56.8
Ash, % DM	12.6	9.60
Crude protein, % DM	10.5	11.6
Acid detergent fiber, % DM	37.0	29.4
Neutral detergent fiber, % DM	61.1	50.7
Ether extract, % DM	1.98	3.48
Ca, % DM	0.95	0.78
P, % DM	0.40	0.41

<sup>1</sup>From breeding to day 84 of pregnancy, heifers were assigned to one of two nutritional strategies: (LG) a basal total mixed ration formulated to support approximately 0.28 kg of daily weight gain (n = 23), or (MG) the same basal ration combined with an energy-protein supplement designed to promote a gain close to 0.79 kg/d (n = 25). <sup>2</sup>The supplemental blend consisted of ground corn, dried distillers grains with solubles (DDGS), wheat middlings, fish oil, urea, and ethoxyquin, and was administered at 0.58% of body weight.

were managed as a single group until parturition, and F0 dam/F1 offspring pairs were collectively raised on pasture until weaning of F1 heifers and their subsequent transport back to the BCRC. A more detailed description of the treatments and management of the F0 dams is reported in Baumgaertner et al. (2024a).

At BCRC, during the growing period, F1 heifers had *ad libitum* access to a forage-based TMR consisting of 70% prairie grass hay, 20% corn silage, and 10% premix on DM basis (Table 2). It is important to note that F1 heifers were managed similarly regardless of the F0 maternal treatments to investigate potential multigeneration effects. The feeding behavior and performance results of F1 heifers during the growing period until the onset of reproduction were reported by Baumgaertner et al. (2024a). At approximately 15 months, F1 heifers were subjected to estrus synchronization and were randomly assigned within each maternal nutritional group to one of two breeding groups due to slaughter facility processing capacity, which ensured that all pregnant heifers were harvested at the same gestational age. A 7-day Select Synch + CIDR estrus synchronization protocol (Lamb et al., 2010) was employed, and timed AI was performed with female sexed semen from a single sire between 48 and 72 hours after CIDR removal based on visual observation of estrus. The use of a single sire to inseminate all F1 heifers was an intentional strategy to reduce paternal genetic variation in the F2 fetuses and allow a more

TABLE 2 Inclusion of ingredients and chemical composition of the diet provided to F1 offspring during the post-weaning period<sup>1</sup>.

Ingredient, % of DM	
Corn silage	20
Prairie hay/winter cereal mix	70
Premix <sup>2</sup>	10
Chemical composition	
DM, %	65.6
Ash, % DM	10.7
Crude protein, % DM	12.3
Acid detergent fiber, % DM	32.9
Neutral detergent fiber, % DM	59.1
Ether extract, % DM	1.44
Ca, % DM	0.66
P, % DM	0.44

<sup>1</sup>F1 offspring were born to dams that were managed for LG (0.28 kg/d; n = 23) or MG (0.79 kg/d; n = 20) during the first 84 d of gestation. F1 offspring had ad libitum access to a common diet during the postweaning development period. <sup>2</sup>The premix was formulated with ground corn, DDGS, a vitamin mineral premix, and monensin.

precise interpretation of the multigenerational effects associated with the maternal nutritional treatments. Nonetheless, we acknowledge that this approach may introduce sire-specific genetic influences, a consideration to be kept in mind when interpreting the results. Heifers not expressing estrus were inseminated again, and fetal sex was determined by ultrasound on day 62 ± 1 after AI (Lamb et al., 2003). To ensure the animals used in molecular analyses were representative of their maternal treatments, eight F1 heifers per treatment were selected to match the average BW of the whole F1 group. The selected heifers averaged LG: 433.9 ± 27.6 kg and MG: 440.1 ± 31.6 kg, while the larger F1 group averaged LG: 442.4 ± 31.9 kg and MG: 451.7 ± 30.3 kg.

For newborn F1 heifers, a sample of approximately 1g of *Longissimus thoracis* muscle (LT) between the 12<sup>th</sup> and 13<sup>th</sup> thoracic vertebrae was collected immediately after calving and before suckling. This region was previously clipped and anesthetized with 2% lidocaine (Lidocaine HCl 2% Injection, MWI, Boise, ID, USA), using a 5 mm external diameter skeletal muscle biopsy needle (Uch Smbn 5Mm X 120Mm Skeletal Musc Biop Ndl, Millennium Surgical, Bala Cynwyd, PA, USA) to extract muscle tissue. At 84 days post-conception (dpc), F1 heifers were euthanized by captive bolt stunning followed by exsanguination at the NDSU Meat Laboratory (Fargo, ND). After hide removal, about 1 g of *Longissimus thoracis* was collected between the 12<sup>th</sup> and 13<sup>th</sup> thoracic vertebrae. For F2 fetuses, the whole fetus was weighed, the viscera removed, and the *Longissimus dorsi* was dissected so that a similar 1 g sample could be taken. All samples collected at F1 birth and those obtained from F1 heifers and F2 fetuses at 84 dpc were placed immediately on dry ice and kept in it throughout the collection period, then transferred to an ultra-freezer at -80°C for storage.

## 2.2 RNA extraction, library preparation, sequencing, and data processing

The RNA extraction from the skeletal muscle of F1 heifers and F2 fetuses was conducted using approximately 30 mg of tissue. Samples were first disrupted and homogenized in TRIzol Reagent (Invitrogen<sup>TM</sup>, Thermo Fisher Scientific, Waltham, MA, USA), and the aqueous phase was then processed using the RNeasy Plus Universal Mini Kit (Qiagen<sup>®</sup>, Germantown, MD, USA) according to the manufacturer's instructions. Sample integrity and purity were assessed using the Agilent 2100 Bioanalyzer and agarose gel electrophoresis. Samples were sequenced only if they met the established quality thresholds and passed Novogene's QC evaluation (RIN value ≥ 5.0, flat baseline, and no detectable degradation or contamination). Overall, the samples exhibited an average RIN of 6.47 ± 1.05. Sixteen samples (8 per treatment) at birth, 15 at the harvest of F1 heifers (LG n = 7; MG n = 8), and 16 samples (8 per treatment) from F2 fetuses met the quantity and quality parameters for library preparation. This process was carried out using the NEBNext<sup>®</sup> Ultra<sup>TM</sup> II non-directional RNA Library Prep Kit for Illumina (New England BioLabs<sup>®</sup>, Ipswich, MA, USA). The libraries were sequenced on the Illumina<sup>®</sup> NovaSeq 6000 platform, generating 150 bp reads at 20 million per sample depth. Both library preparation and paired-end sequencing were conducted by Novogene Co. (Nanjing, China). Data cleaning involved the removal of adapters and reads with a Phred-Score < 30. Quality control (QC) and read statistics were assessed using FastQC v0.11.8 (Andrews, 2023) and MultiQC v1.9 (Ewels et al., 2016). The reads were mapped to the *Bos taurus* reference genome (ARS-UCD 1.2) (Rosen et al., 2020) available at [https://jul2023.archive.ensembl.org/Bos\\_taurus/Info/Index](https://jul2023.archive.ensembl.org/Bos_taurus/Info/Index), using the STAR aligner v. 2.7.3a (Dobin et al., 2013). Raw counts per gene were obtained using the *-quantMode* GeneCounts parameter from STAR and the gene annotation file from Ensembl (release 100). Post-mapping quality control was conducted using the tools MultiQC, NOISeq v.2.26.0 (Tarazona et al., 2015), and edgeR v.3.24.3 (Robinson et al., 2010).

## 2.3 Differential expression and enrichment analyses

Genes non-expressed or lowly expressed (10 counts per million in 70% of samples) were filtered out using the filterbyexpression function from edgeR R-package. After filtering, differentially expressed genes (DEGs) were identified using the DESeq2 v.1.22.1 R package (Love et al., 2014). The DESeq2 median of ratios method was employed to normalize sequencing depth and RNA composition data (Love et al., 2014). Differentially expressed genes were identified using a *p*-value threshold of ≤ 0.05 and an absolute log<sub>2</sub> fold change greater than 0.5 as the criteria for significance and were classified as up-regulated or down-regulated based on the sign of the log<sub>2</sub> fold change in the MG group.

Additionally, all genes tested for differential expression were ranked using the following equation: rank = [sign(log<sub>2</sub>FC) × -log<sub>10</sub>

( $p$  – value)] with the sign determined by the fold-change and the magnitude by the  $p$ -value (Ziemann, 2016). Significant results were identified after multiple testing corrections using an FDR-adjusted  $p$ -value ( $\leq 0.05$ ), with *Bos taurus* annotation as the reference.

Functional enrichment analysis of the ranked gene list was performed in WebGestalt (Liao et al., 2019) using Gene Set Enrichment Analysis (GSEA). Gene sets had to contain between 5 and 2,000 genes. Enrichment scores were obtained from 1,000 permutations, and the normalized enrichment score (NES) was used to interpret the direction and strength of enrichment. A positive NES indicates that the gene set is enriched toward the top of the ranked list, where genes with positive fold changes predominate, whereas a negative NES reflects enrichment toward the bottom of the list, where genes with negative fold changes are more frequent. GO Biological Process and KEGG categories were considered significantly enriched when they met the FDR  $\leq 0.05$  criterion, and both positive and negative enrichments were reported. For visualization purposes, only the ten most enriched categories in each direction were presented.

### 3 Results

Differentially expressed genes were identified in *Longissimus thoracis* (LT) muscle samples from F1 heifers at birth and harvest and F2 fetuses at 84 dpc, influenced by the rate of body weight gain of F0 dams during early gestation. Using RNA-Seq, an average of 26.7M clean reads per sample (20.0 to 45.2M) were generated across 48 samples, equally divided among F1 heifers at birth, at harvest, and F2 fetuses. The sequencing yield and mapping rates for each phase and sample in F1 heifers and F2 fetuses are detailed in Supplementary Table S1. On average, 91.8%, 91.9%, and 92.3% of LT muscle reads from F1 heifers at birth, harvest, and F2 fetuses at 84 dpc were uniquely mapped to genes in the bovine reference genome. After filtering, 12,485, 12,488, and 14,200 genes from the LT muscle of F1 heifers at birth, harvest, and F2 fetuses at 84 dpc were analyzed to identify DEGs.

#### 3.1 Differentially expressed genes in *Longissimus thoracis* muscle of F1 heifers and F2 fetuses

At birth, 275 DEGs were identified, with 177 being upregulated and 98 downregulated in F1 offspring born to MG dams compared to the LG group (Figure 1). At harvest, 177 DEGs were identified in the same F1 heifers, of which 75 were upregulated and 102 downregulated (Figure 1). From F2 fetuses, 151 DEGs were identified, with 63 upregulated and 88 downregulated in the MG group (Figure 1).

An overlap of DEGs between MG vs. LG identified in F1 heifers at birth, at harvest, and in F2 fetuses at 84 dpc was conducted using the Venny 2.1 web tool (Oliveros, 2007). Eleven DEGs (Figure 2) were common for F1 heifers at birth and harvest, with eight genes downregulated at both time points (*ARG2*, *ASNS*, *PCK2*, *PSPH*,

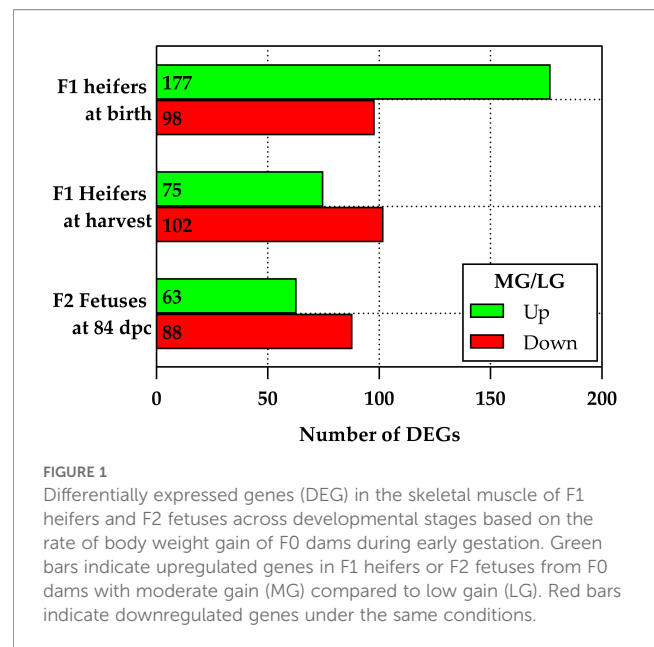


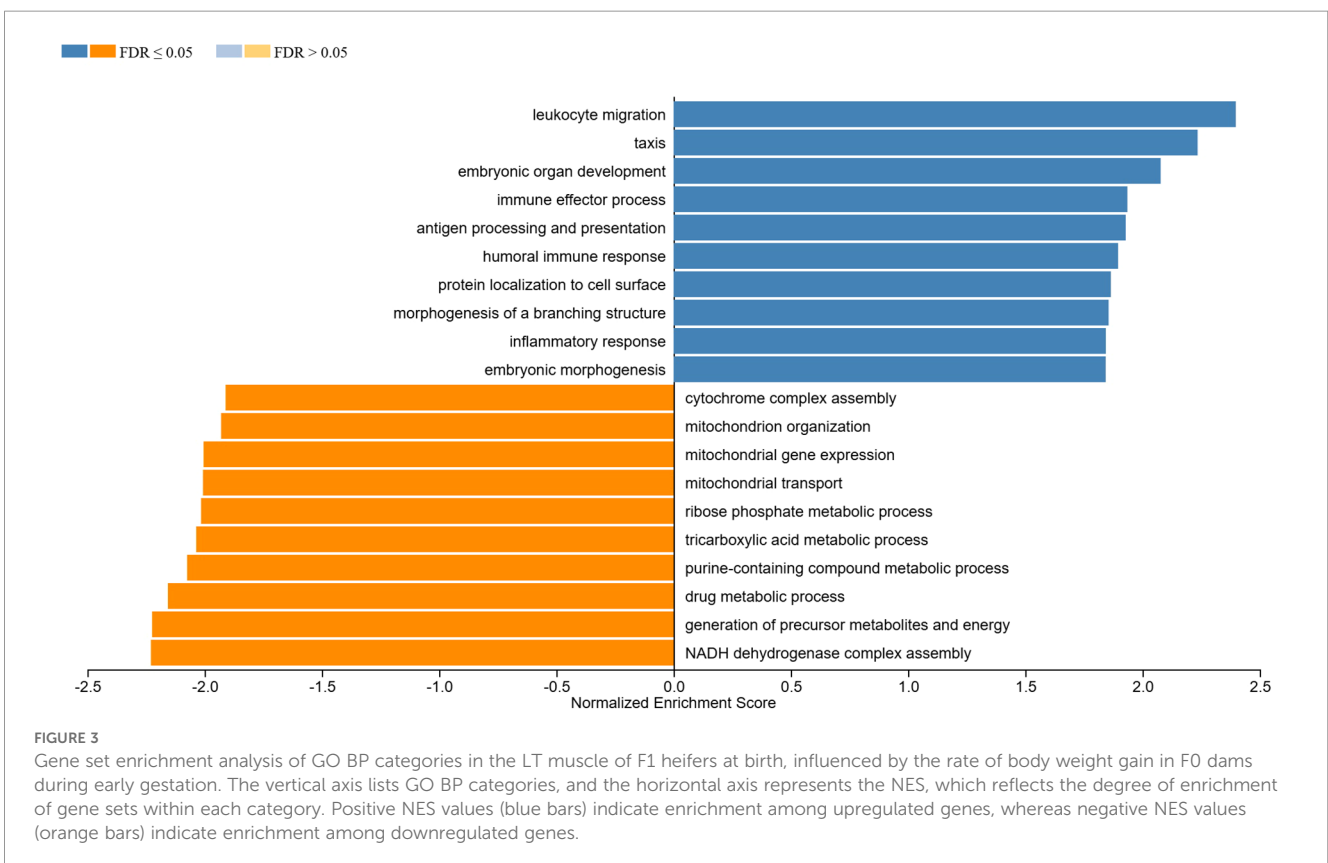
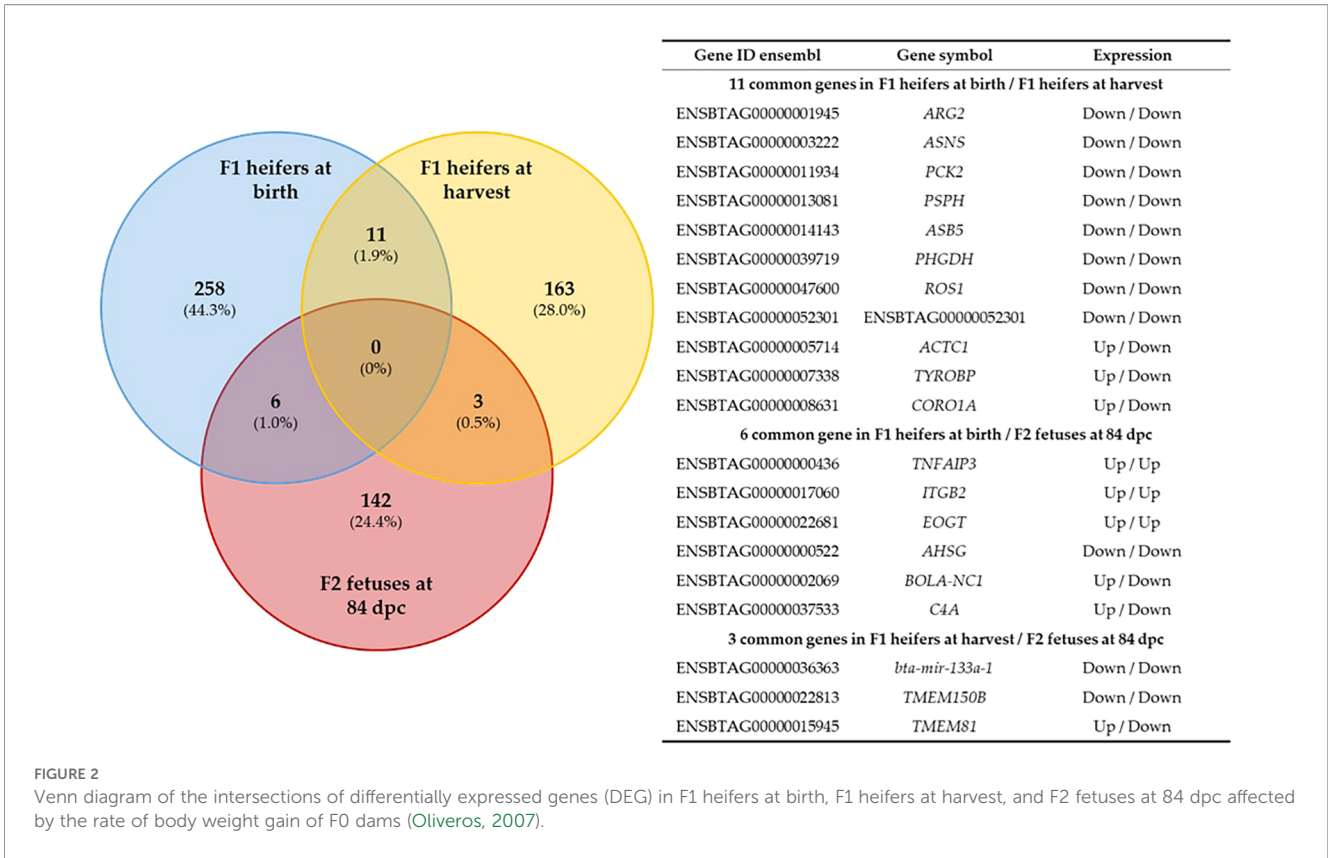
FIGURE 1

Differentially expressed genes (DEG) in the skeletal muscle of F1 heifers and F2 fetuses across developmental stages based on the rate of body weight gain of F0 dams during early gestation. Green bars indicate upregulated genes in F1 heifers or F2 fetuses from F0 dams with moderate gain (MG) compared to low gain (LG). Red bars indicate downregulated genes under the same conditions.

*ASB5*, *PHGDH*, *ROS1*, *ENSBTAG00000052301*) and three upregulated at birth and downregulated at harvest (*ACTC1*, *TYROBP*, *CORO1A*). An overlap of DEGs between F1 heifers at birth and F2 fetuses at 84 dpc revealed six common DEGs. Among them, *TNFAIP3*, *ITGB2*, and *EOGT* were upregulated in F1 heifers at birth, while *AHSG* was downregulated in F2 fetuses. The *BOLA-NC1* and *C4A* genes were upregulated in F1 heifers at birth and downregulated in F2 fetuses, respectively. Among the LT samples from F1 heifers at harvest and F2 fetuses at 84 dpc, three DEGs were identified, with *bta-mir-133a-1* and *TMEM150B* downregulated, and *TMEM81* upregulated at harvest and downregulated in F2 fetuses. No overlap was observed between all the three time points. Fold change values for each common DEGs are detailed in Supplementary Tables S2–S4.

#### 3.2 Functional enrichment analysis of DEGs in F1 heifers and F2 fetuses

Among the 275 DEGs identified in the LT muscle of F1 heifers at birth, considering the 12,485 genes evaluated in the GSEA, several GO BP categories showed significant enrichment based on the FDR  $\leq 0.05$  threshold. The set of 177 upregulated DEGs contributed to the enrichment of processes with positive NES values, primarily those related to the immune system, such as leukocyte migration (GO:0050900), taxis (GO:0042330), immune effector process (GO:0002252), antigen processing and presentation (GO:0019882), humoral immune response (GO:0006959), protein localization to cell surface (GO:0034394), and inflammatory response (GO:0006954) (blue bars; Figure 3). Additionally, processes associated with embryonic development, including embryonic organ development (GO:0048568), morphogenesis of a branching structure (GO:0001763), and embryonic morphogenesis (GO:0048598), also showed significant enrichment with positive



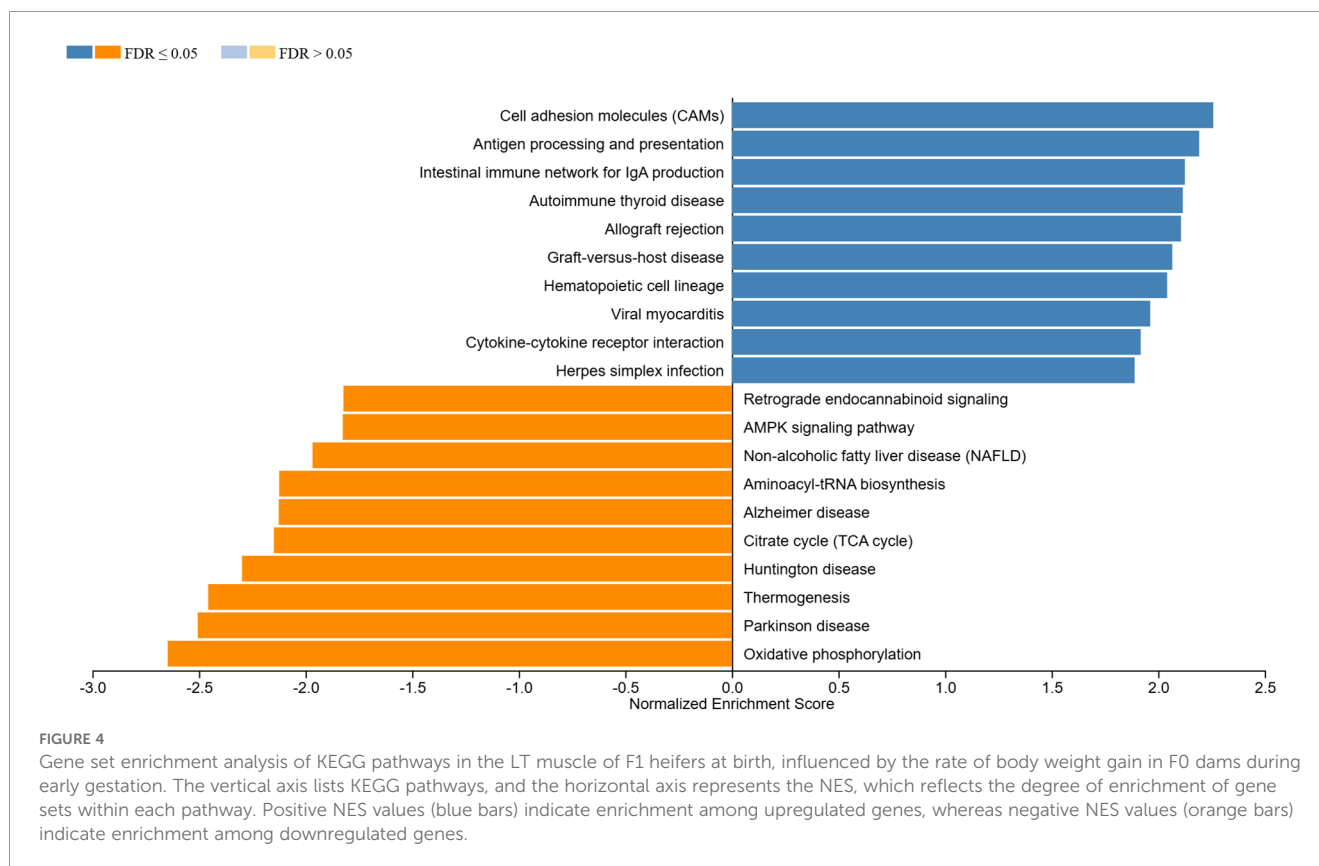
NES values in F1 MG heifers at birth. In contrast, the 98 downregulated DEGs contributed to GO BP categories that showed significant enrichment with negative NES values, including processes associated with mitochondrial function, such as cytochrome complex assembly (GO:0017004), mitochondrion organization (GO:0007005), mitochondrial gene expression (GO:0140053), and mitochondrial transport (GO:0006839), as well as central metabolic pathways, including tricarboxylic acid metabolic process (GO:0072350), generation of precursor metabolites and energy (GO:0006091), NADH dehydrogenase complex assembly (GO:0010257), ribose phosphate metabolic process (GO:0019693), and purine-containing compound metabolic process (GO:0072521) (orange bars; Figure 3).

The KEGG pathway enrichment analysis showed a similar pattern. Pathways associated with the 177 upregulated DEGs showed significant enrichment, as indicated by positive NES values, for immune-related processes, including antigen processing and presentation (bta04612), intestinal immune network for IgA production (bta04672), graft-versus-host disease (bta05332), and cytokine–cytokine receptor interaction (bta04060). Conversely, pathways associated with the 98 downregulated DEGs showed significant enrichment with negative NES values, including AMPK signaling pathway (bta04152), citrate cycle (TCA cycle) (bta00020), oxidative phosphorylation (bta00190), aminoacyl-tRNA biosynthesis (bta00970), and thermogenesis (bta04714) (Figure 4).

Of the 177 DEGs identified in F1 heifers at harvest from F0 MG dams compared with the LG group, no positively regulated GO biological process terms reached statistical significance in the enrichment analysis ( $FDR > 0.05$ ; Figure 5). In contrast, enrichment analysis revealed negatively regulated GO BP terms in F1 heifers from MG dams, encompassing categories related to energy metabolism, such as generation of precursor metabolites and energy (GO:0006091) and tricarboxylic acid metabolic process (GO:0072350), as well as processes associated with protein and amino acid metabolism, including cellular amino acid metabolic process (GO:0006520) and protein folding (GO:0006457). Processes involved in nucleic acid metabolism, such as nucleobase-containing small molecule metabolic process (GO:0055086), were also enriched among these negatively regulated processes (orange bars; Figure 5).

The KEGG pathway enrichment analysis performed with the 177 identified DEGs showed that the positively regulated pathways were not significantly affected by the moderate rate of body weight gain in F0 dams in F1 heifers at harvest ( $FDR > 0.05$ ; Figure 6). In contrast, negatively regulated pathways that play essential roles in protein homeostasis and cellular energy production were significantly enriched. Among these downregulated pathways were aminoacyl-tRNA biosynthesis (bta00970), ribosome (bta03010), biosynthesis of amino acids (bta01230), proteasome (bta03050), and oxidative phosphorylation (bta00190) (Figure 6).

Regarding F2 fetuses influenced by the rate of body weight gain of F0 dams, the enrichment analysis performed using GSEA,



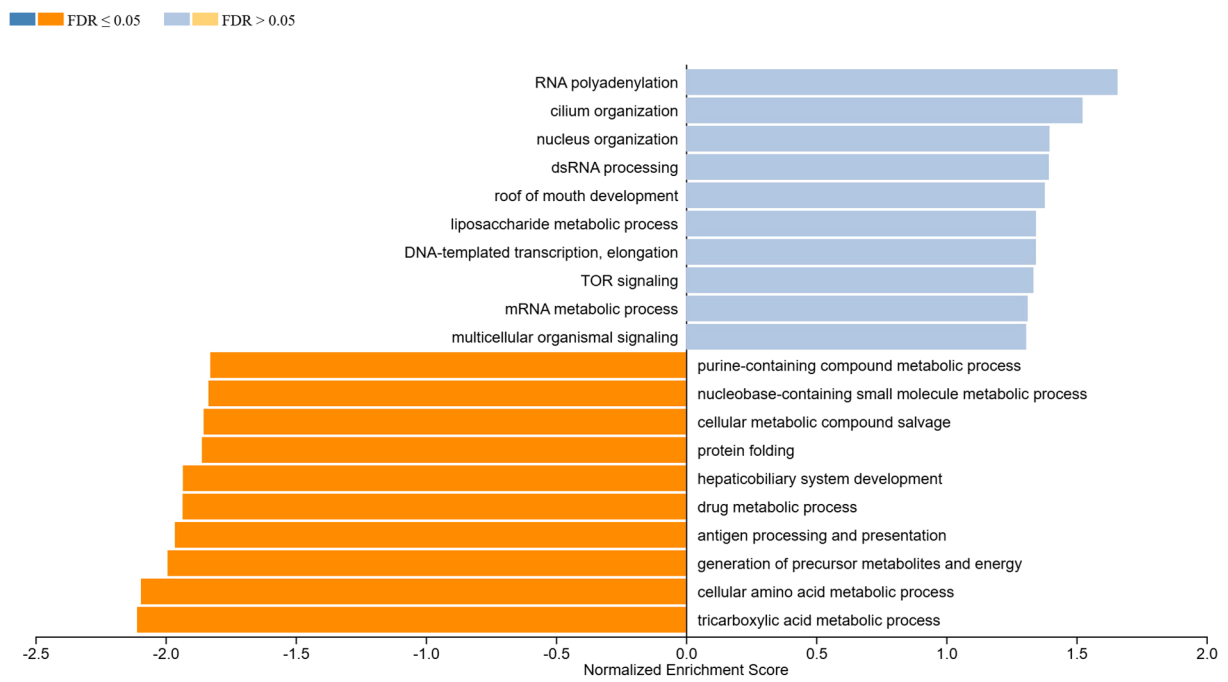


FIGURE 5

Gene set enrichment analysis of GO BP categories in the LT muscle of F1 heifers harvested at 84 days of gestation, influenced by the rate of body weight gain in F0 dams during early gestation. The vertical axis lists GO BP categories, and the horizontal axis represents the NES, which reflects the degree of enrichment of gene sets within each category. Positive NES values (blue bars) indicate enrichment among upregulated genes, whereas negative NES values (orange bars) indicate enrichment among downregulated genes.

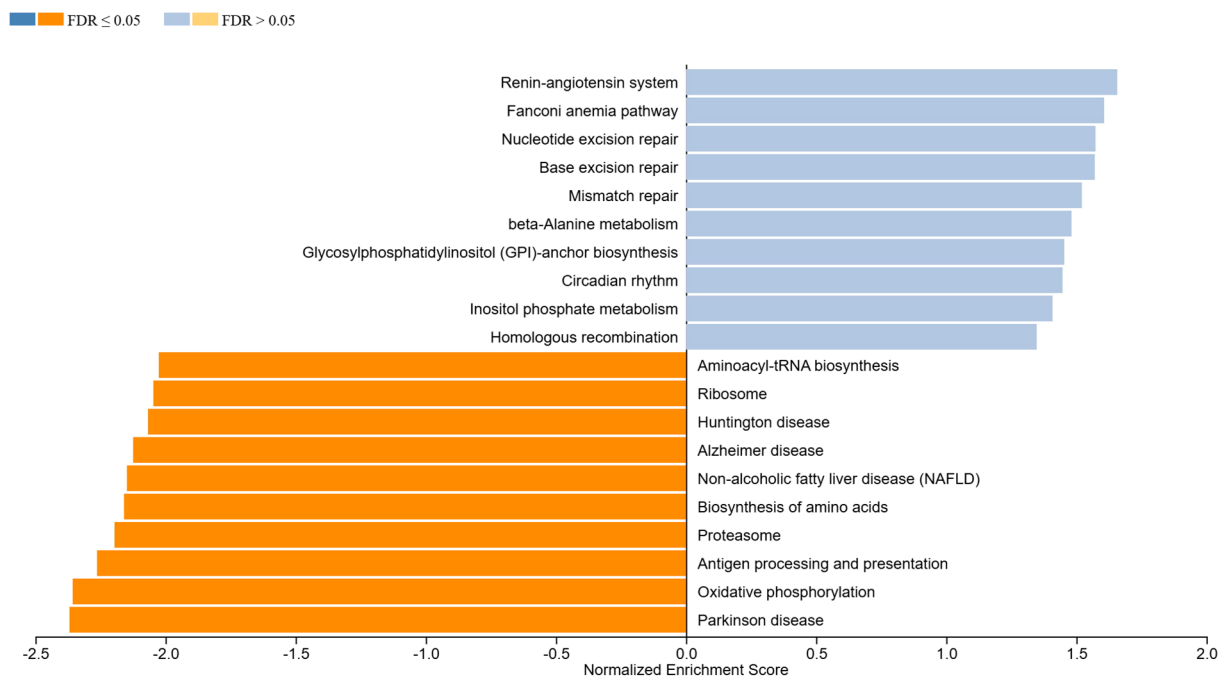
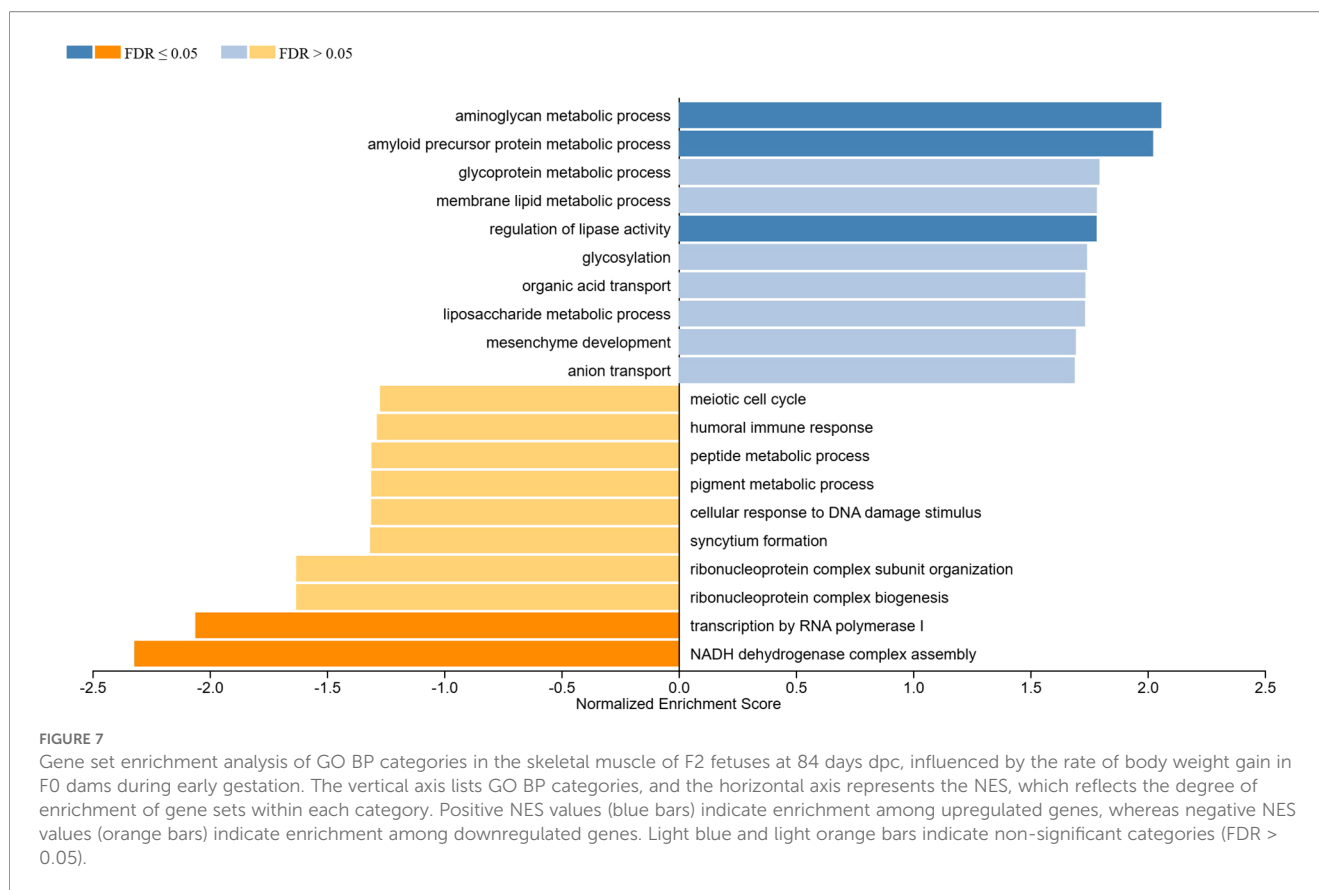


FIGURE 6

Gene set enrichment analysis of KEGG pathways in the LT muscle of F1 heifers harvested at 84 days of gestation, influenced by the rate of body weight gain in F0 dams during early gestation. The vertical axis lists KEGG pathways, and the horizontal axis represents the NES, which reflects the degree of enrichment of gene sets within each pathway. Positive NES values (blue bars) indicate enrichment among upregulated genes, whereas negative NES values (orange bars) indicate enrichment among downregulated genes.



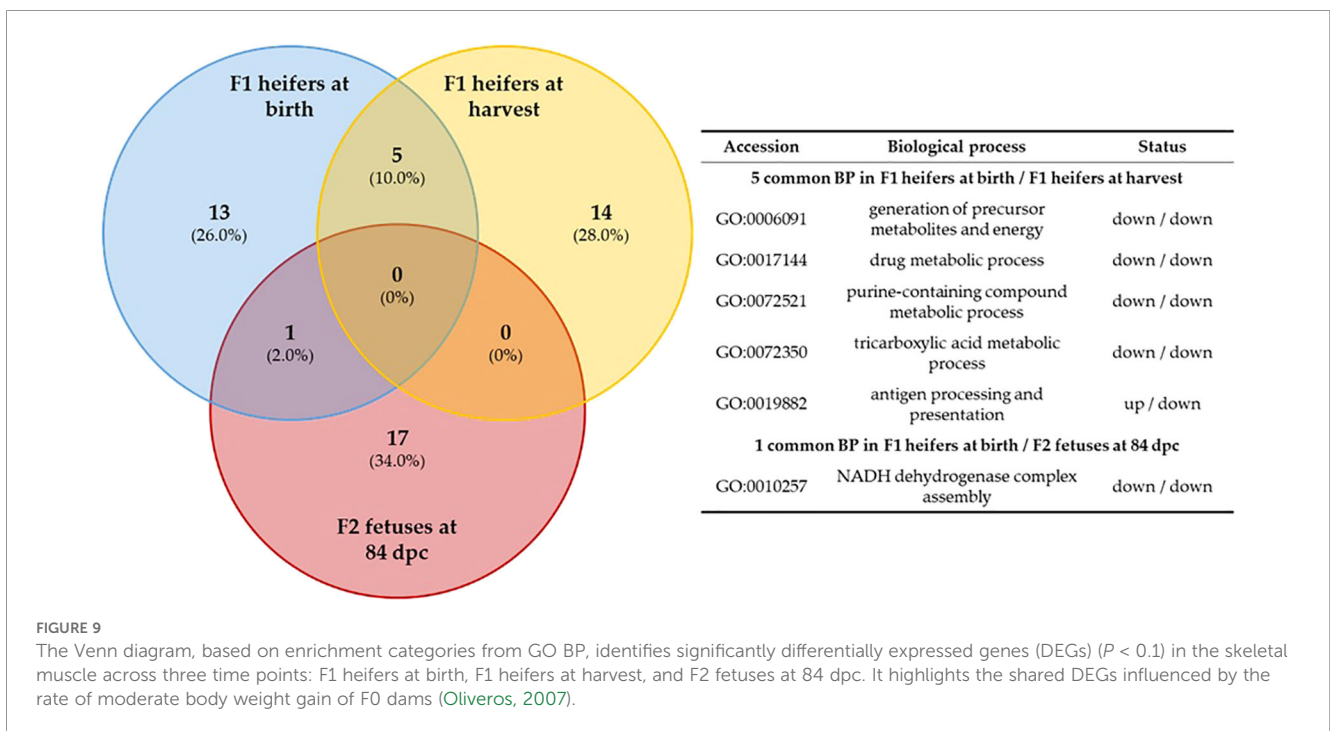
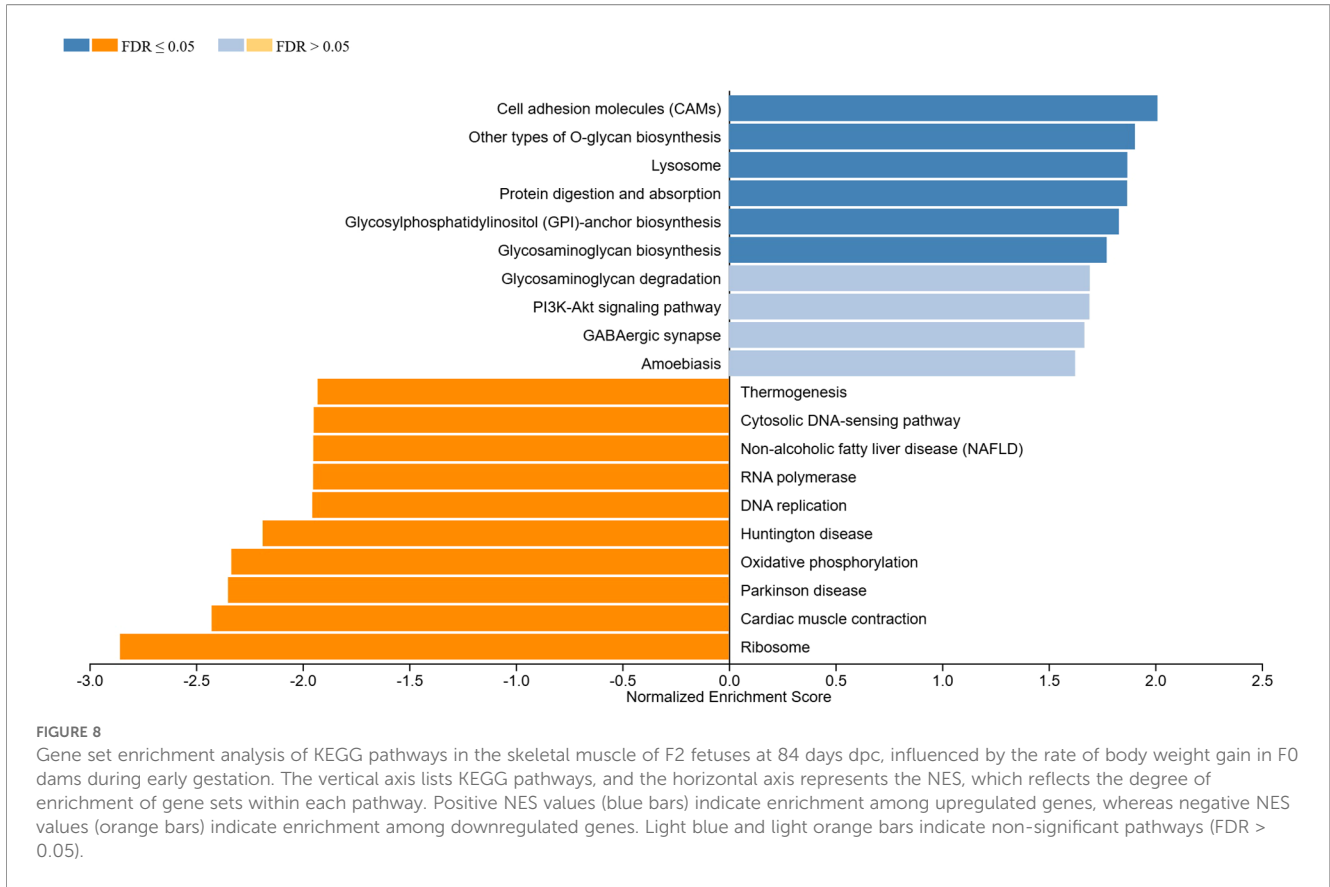
conducted using a total of 14,200 genes, including the 151 DEGs identified at 84 dpc, revealed GO BP terms in the skeletal muscle of F2 fetuses from F0 MG dams compared to those from F0 LG dams. This analysis highlighted biological processes such as aminoglycan metabolic process (GO:0006022), amyloid precursor protein metabolic process (GO:0042982), and regulation of lipase activity (GO:0060191). Although other GO BP categories with positive NES values were not significant after FDR correction (FDR  $\geq$  0.05; Figure 7), the following can be cautiously highlighted ( $P \leq$  0.029; FDR  $\leq$  0.068; Supplementary Table S9): glycoprotein metabolic process (GO:0009100), membrane lipid metabolic process (GO:0006643), organic acid transport (GO:0015849), mesenchyme development (GO:0060485), and glycosylation (GO:0070085). On the other hand, in the same group of F2 MG fetuses, GO BP terms such as transcription by RNA polymerase I (GO:0006360) and NADH dehydrogenase complex assembly (GO:0010257) showed negative NES values, indicating lower relative enrichment in the ranked gene list.

In F2 MG fetuses, the KEGG pathways enriched by the DEGs showed significant enrichment with positive NES values, highlighting their importance in cell adhesion, protein modification and function, cellular recycling, and signaling (Figure 8). The enriched pathways include cell adhesion molecules (CAMs) (bta04514), other types of O-glycan biosynthesis (bta00514), lysosome (bta04142), protein digestion and absorption (bta04974), glycosylphosphatidylinositol (GPI)-anchor biosynthesis (bta00563), and glycosaminoglycan

biosynthesis (bta00532). Additionally, the PI3K-Akt signaling pathway (bta04151), although not significantly enriched ( $P <$  0.001; FDR = 0.098; Supplementary Table S10), plays a crucial role in regulating growth factors and cell proliferation. In contrast, several cellular function and homeostasis exhibited negative NES values in F2 MG fetuses compared to those from F0 LG dams. These include thermogenesis (bta04714), cytosolic DNA-sensing pathway (bta04623), RNA polymerase (bta03020), DNA replication (bta03030), oxidative phosphorylation (bta00190), and ribosome (bta03010).

Overlap analysis of significantly enriched GO Biological Process terms identified in F1 heifers at birth, F1 heifers at harvest, and F2 fetuses at 84 dpc in the MG group compared with the LG group (Figure 9). Positively regulated processes include antigen processing and presentation (GO:0019882), which were upregulated at birth and downregulated at harvest in F1 heifers. Negatively regulated overlapping processes included the generation of precursor metabolites and energy (GO:0006091), drug metabolic process (GO:0017144), purine-containing compound metabolic process (GO:0072521), and tricarboxylic acid metabolic process (GO:0072350), which were consistently downregulated in the MG group at both birth and harvest in F1 heifers. Additionally, NADH dehydrogenase complex assembly (GO:0010257) was negatively regulated in both F1 heifers at birth and F2 fetuses at 84 dpc.

In the KEGG pathway overlap analysis, significant regulatory patterns were observed among F1 heifers at birth and at harvest, and among F2 fetuses at 84 dpc, within the MG group compared



with the LG group (Figure 10). Three common KEGG pathways were identified between F1 heifers at birth and at harvest, including Alzheimer disease (bta05010) and Aminoacyl-tRNA biosynthesis (bta00970), both of which were negatively regulated in the MG group at both time points. In contrast, Antigen processing and presentation (bta04612) was positively regulated at birth and negatively regulated at harvest in the MG group. Two common KEGG pathways were identified between F1 heifers at birth and F2 fetuses at 84 dpc: Thermogenesis (bta04714) was negatively regulated in the MG group in both sets, whereas Cell adhesion molecules (CAMs) (bta04514) were positively regulated in both sets. When comparing the KEGG pathways of F1 heifers at harvest with those of F2 fetuses at 84 dpc, one common pathway was identified: Ribosome (bta03010), which was negatively regulated in the MG group in both sets. Finally, four common KEGG pathways were identified among F1 heifers at birth and harvest and F2 fetuses at 84 dpc, including Oxidative phosphorylation (bta00190), Parkinson disease (bta05012), Huntington disease (bta05016), and Non-alcoholic fatty liver disease (NAFLD) (bta04932), all of which were consistently negatively regulated in the MG group across the three sets.

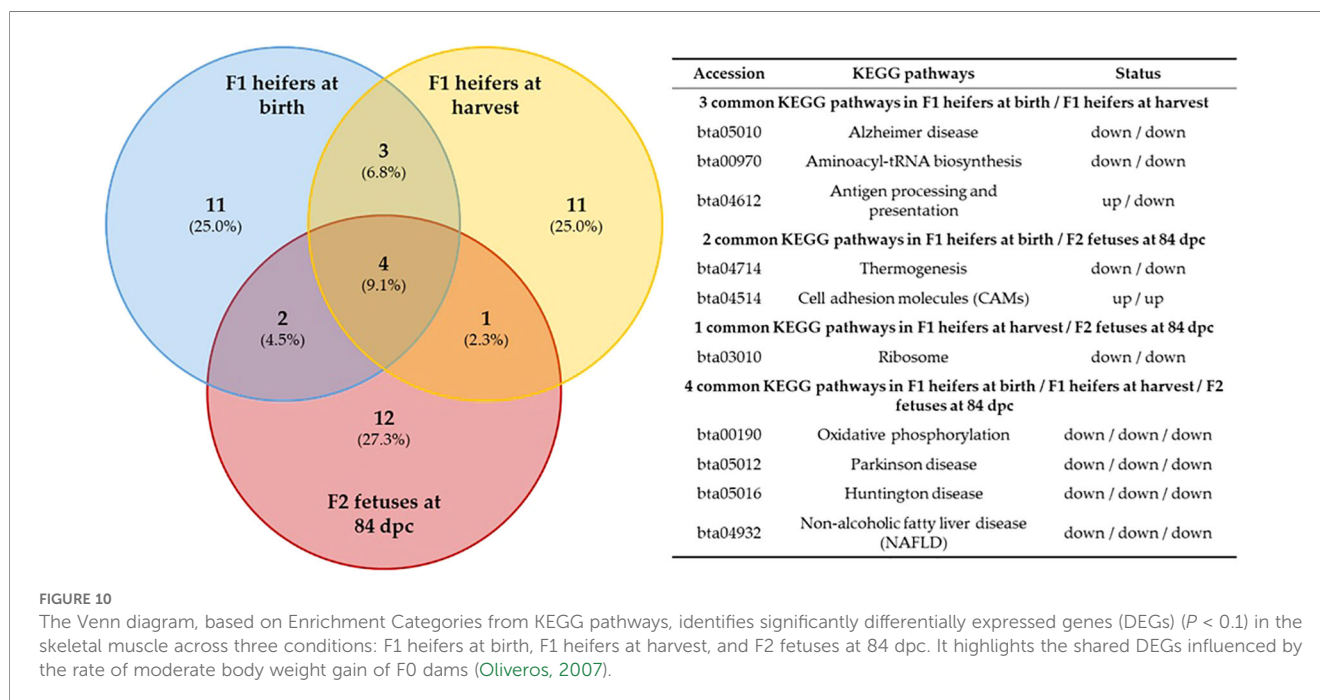
## 4 Discussion

Our nutritional model, provides evidence of nutritional effects on maternal and fetal development. These responses were particularly evident as F1 offspring of MG dams were heavier at birth and during the post-weaning period (Baumgaertner et al., 2024a). Although the first trimester is not characterized by significant muscle fiber formation, it establishes the foundation for future myogenic activity (Picard and Gagaoua, 2020). We identified 603 DEGs across all time points in our gene expression

analyses, with differential expression patterns interpreted based on the MG versus LG comparison. Accordingly, whenever opposite regulation is described, it refers to the LG group relative to MG, in order to illustrate the biological differences between the groups and to facilitate biological interpretation. This approach highlights the compensatory mechanisms and metabolic shifts that distinguish muscle development between MG and LG F1 heifers and F2 fetuses.

### 4.1 Impact of F0 dam rate of body weight gain during early gestation on gene regulation and biological and metabolic enrichment in the muscle of F1 offspring

Environmental or nutritional changes can reprogram fetal muscle development during periods when it remains plastic (Hocquette, 2010; Aragão et al., 2014; Shokrollahi et al., 2024b). To date, the effects of maternal nutrition on fetal muscle have been investigated mainly from a structural perspective (Costa et al., 2021; Greenwood and Café, 2007; Marquez et al., 2017; Maresca et al., 2019; Nascimento et al., 2024). During prenatal development, skeletal muscle fiber metabolism is a programmed process detectable in ruminants as early as 30 days post-conception (Bonnet et al., 2010). To sustain these processes, the fetus relies primarily on carbohydrates and amino acids as energy sources for oxidative metabolism, protein synthesis, and the structural organization of muscle (Battaglia and Meschia, 1978). During gestation, substrate partitioning is primarily regulated by the demands of the placenta and fetal organs, which conditions the systemic availability of nutrients to skeletal muscle. Complementary studies to the present work published by our group, using differential gene expression analysis in placentomes (Diniz et al., 2021) and lipidomics in fetal liver (Menezes et al., 2023) at 83 days



of gestation, demonstrated positive regulation of genes (*SREBF2*, *FDFT1*, *FADS1*, *MSMO1*, and *SQLE*) related to fatty acid metabolism in placentomes of F0 dams from the LG group compared with the MG group. In the lipidomic analysis, 152 of 374 metabolites and 32 of 57 identified pathways in the fetal liver showed a greater response in F1 fetuses from the LG group compared with the MG group, indicating a preferential redirection of fetal hepatic metabolism toward lipid pathways, despite the fact that F0 dams from the MG group received a lipid-rich supplement composed of fish oil and dried distillers grains with solubles.

At birth and persisting during postnatal life (at harvest), the *PCK2* gene was upregulated in the muscle of F1 LG heifers compared with the MG group. This gene encodes the PEPCK-M, an enzyme with a well-established role in intermediary metabolism, primarily characterized in hepatic tissue (Fassah et al., 2018). Supporting a role for *PCK2* in metabolic flexibility, studies in tumor cells have demonstrated that PEPCK-M promotes the incorporation of carbons derived from oxaloacetate into serine and glycine biosynthesis (Vincent et al., 2015), highlighting its importance in sustaining biosynthetic demands under metabolically unfavorable conditions (Méndez-Lucas et al., 2014; Vincent et al., 2015). Consistently, in porcine skeletal muscle, *PCK2* expression has been associated with the coordinated induction of genes involved in the serine biosynthesis pathway, including *PHGDH*, *PSAT1*, and *PSPH* (Brown et al., 2016). In the present study, we observed upregulation of *PHGDH* and *PSPH* at both birth and harvest in F1 heifers from the LG group, whereas *PSAT1* was upregulated only at harvest, compared with the MG group (Supplementary Table S3). This gene set showed enrichment for the cellular amino acid metabolic process (GO:0006520) and the biosynthesis of amino acids pathway (bta01230) in the muscle of F1 LG heifers at harvest, in agreement with the negative enrichment observed in the MG group. Evidence from tumor cells (Locasale et al., 2011; Possemato et al., 2011; DeNicola et al., 2015) and highly proliferative embryonic progenitors (Tedeschi et al., 2013) indicates that the expression of *PHGDH*, *PSAT1*, and *PSPH* decreases as cells enter differentiation. Consistently, a study with C2C12 muscle cells found that the relative expression of *PCK2*, *PHGDH*, *PSAT1*, *PSPH*, and *ASNS* decreases when these cells enter the differentiation phase into myotubes (Brearley et al., 2019).

During myogenesis in cattle, a coordinated multi-step process, the hyperplasia phase ends at approximately 180 days of gestation (Bonnet et al., 2010; Du et al., 2010; Santos et al., 2022). After this period, muscle growth predominantly occurs through hypertrophy, mediated by the proliferation and fusion of satellite cells with pre-existing muscle fibers (Picard and Gagaoua, 2020), as well as increased protein synthesis rates (Brearley et al., 2019). These events impose high metabolic demands on skeletal muscle, and the upregulation of *PCK2*, *PHGDH*, *PSAT1*, *PSPH*, and *ASNS* in F1 heifers from the LG group compared with the MG group may be associated with a compensatory response mediated by satellite cells, supporting hypertrophic growth of adult muscle.

To corroborate this hypothesis, we observed downregulation of *miR-1* at birth and upregulation of *bta-miR-133a-1* at harvest in F1

heifers from the LG group compared with the MG group. *miR-1* and *miR-133* exert distinct roles in gene regulation in cultured myoblasts, modulating both proliferation and differentiation in skeletal muscle (Sun et al., 2013). By binding to *HDAC4* mRNA, *miR-1* promotes myogenic gene expression, accelerating differentiation and reducing cellular proliferation (Rosenberg et al., 2006; Chen et al., 2006). In satellite cells, *miR-1* also significantly reduces proliferation by inhibiting *Pax7* (Chen et al., 2010). Although *HDAC4* and *Pax7* were not detected among the DEGs in skeletal muscle in the present study, the downregulation of *miR-1* at birth in F1 LG heifers may suggest the maintenance of a proliferative state in these cells, favoring the expansion of the pool of myogenic precursors. In contrast, the upregulation of *bta-miR-133a-1* at harvest in the LG group may indicate the consolidation of differentiation of these cells toward muscular hypertrophy, since this miRNA directly controls the *MyoD*-differentiation axis in satellite cells (Zammit et al., 2006; Rhoads et al., 2009). In addition, *ASB5*, recently associated with muscle differentiation in cattle (Schettini et al., 2022), was upregulated at harvest in F1 LG heifers compared with the MG group, suggesting satellite cell fusion with pre-existing muscle fibers during hypertrophy. Although these molecular patterns suggest adaptations compatible with this scenario of muscle growth and metabolic reorganization, additional studies integrating muscle fiber characteristics, skeletal muscle metabolism, and gene expression profiles are still required to confirm this interpretation.

In cattle, the *ACTC1* gene is expressed during the final stages of myogenic differentiation, culminating in myotube formation (Li et al., 2021). In our study, the downregulation at birth and the upregulation at harvest of this gene in F1 heifers from the LG group relative to the MG group may be associated with temporal responses of proliferation followed by satellite cell differentiation, since *ACTC1* modulation contributes significantly to the final stages of differentiation of these cells (Chen et al., 2023). In this context, the reduced expression of *ACTC1* at birth in F1 LG heifers compared with the MG group, together with the previously observed downregulation of *miR-1* in this same group, may be compatible with the preservation of a broader pool of myogenic precursor cells, may potentially favor compensatory hypertrophic responses during postnatal growth and adulthood. This interpretation is supported by the upregulation of *bta-miR-133a-1* at harvest in F1 LG heifers relative to the MG group, which may be associated with the differentiation of these cells during hypertrophic processes.

A similar pattern, characterized by lower abundance of *miR-1* and *miR-133a* in the skeletal muscle of offspring from cows subjected to energy restriction during mid-gestation, was described by Paradis et al. (2017) and interpreted by the authors as indicative of delayed muscle differentiation. However, unlike our study, the gestational window evaluated by these authors coincided with the phase of secondary muscle fiber formation, with fetal sampling performed at approximately 247 days of gestation. Considering that the maternal nutritional intervention in our study was applied during early gestation, a period corresponding predominantly to primary myogenesis, a potential reduction in

primary fiber formation could, in theory, subsequently limit the number of secondary fibers, since primary fibers act as a structural scaffold for the organization of secondary fibers (Du et al., 2010). Under favorable nutritional conditions, after the critical period of muscle hyperplasia, tissue growth occurs mainly through hypertrophy, with active satellite cells as the main contributors (Picard and Gagaoua, 2020). Satellite cells, detectable as early as two months of gestation in bovine fetuses (Russell and Oteruelo, 1981), continue to proliferate during subsequent peri- and postnatal stages (Biressi et al., 2007; Dumont et al., 2015). In this context, it is plausible that the nutritional intervention during early gestation in our study did not produce sufficiently severe effects on primary fibers to directly affect secondary fiber formation or the dynamics of the satellite cell population.

Furthermore, since muscle sampling of the F1 offspring was performed during postnatal life, the lack of detection of canonical fetal myogenesis genes, such as *Pax3*, *Pax7*, and the MRFs, as well as biological processes and classical pathways directly associated with myogenesis, may be related not only to the gestational window of the nutritional intervention in F0 dams but also to the time elapsed between the nutritional event and sample collection. In this scenario, early alterations in myogenic development may have been reorganized across subsequent fetal and postnatal stages, manifesting primarily through mechanisms associated with satellite cell function and muscle mass maintenance. Thus, the modulation of genes such as *ACTC1* and miRNAs such as *miR-1* and *miR-133a* appears to be more closely related to satellite cell activity than to the central muscle cells that compose the fibers.

Baumgaertner et al. (2024a), in a complementary study with a larger cohort of F0 dams, observed that F1 heifers from the LG group were about 2 kg lighter at birth compared with those from the MG group. After birth, both progenies were raised under the same nutritional conditions and showed no differences in body weight until weaning. From 14 months of age onward, however, the LG group had lower body weight than the MG group, whereas no differences were observed at slaughter or at 84 days of gestation (Baumgaertner et al., 2024b). Additionally, no differences were detected in *Longissimus* muscle area between groups, either at 69 days post-weaning (LG = 49.2 cm<sup>2</sup> vs. MG = 50.3 cm<sup>2</sup>; *P* = 0.53; Baumgaertner et al., 2024a) or at harvest (LG = 61.9 cm<sup>2</sup> vs. MG = 61.8 cm<sup>2</sup>; *P* = 0.96; Baumgaertner et al., 2024b). These results suggest that subtle adjustments in compensatory mechanisms associated with muscle hypertrophy may have been activated in F1 heifers from the LG group, preserving the *Longissimus* muscle area at levels similar to those in the MG group. Although our study did not identify direct changes in genes, biological processes, or classical pathways associated with fetal myogenesis, molecular changes in biological processes and metabolic pathways required for muscle maintenance were observed.

Studies in muscle cells indicate that oxidative metabolism progressively becomes the primary energy pathway as differentiation progresses, largely due to increased mitochondrial remodeling (Wagatsuma and Sakuma, 2013; Sin et al., 2016). In the present study, skeletal muscle collected at birth showed greater functional representation of GO BP terms related to cytochrome

complex assembly, mitochondrial organization, mitochondrial gene expression, and mitochondrial transport in F1 LG heifers compared with the MG group. At face value, the enrichment of these biological processes linked to mitochondrial biogenesis could suggest a more advanced differentiation state in LG heifers at birth, given that the metabolic shift from glycolysis to oxidative phosphorylation is a hallmark of myogenic differentiation (Herzberg et al., 1993; Korohoda et al., 1993; Pawlikowska et al., 2006). Nevertheless, experimental studies conducted under conditions of muscle repair and regeneration demonstrate that mitochondrial remodeling is also required to meet the energetic demands of both satellite cell proliferation and differentiation (Buas and Kadesch, 2010; Jash and Adhya, 2012). Therefore, the enrichment of these pathways in F1 LG heifers at birth may reflect, at least in part, a metabolic support for satellite cell activity rather than exclusively indicating advanced myogenic maturation.

In parallel, the positive regulation of *ARG2* in F1 LG heifers relative to the MG group, together with enrichment of mitochondrial biogenesis and oxidative phosphorylation-related pathways, is consistent with metabolic adjustments that favor greater reliance on oxidative metabolism. Given the marked metabolic plasticity of skeletal muscle during early postnatal life, this response may create a permissive environment for sustained satellite cell activity and, potentially, for subsequent adjustments in muscle fiber composition. In this context, *ARG2* is particularly relevant because its encoded enzymes are essential for nitric oxide production, which promotes vasodilation and increased blood flow in skeletal muscle, facilitating fatty acid uptake and oxidation (Jobgen et al., 2006), in addition to modulating the activation of resident satellite cells (Anderson and Pilipowicz, 2002). Lower circulating glucose concentrations reported in F1 LG heifers (Baumgaertner et al., 2024b), together with the upregulation of *PCK2* in this group, support the interpretation of a metabolic adjustment aimed at preserving muscle glucose availability through the use of alternative substrates. In this scenario, the upregulation of *ARG2* in F1 LG heifers may be interpreted as part of a broader metabolic plasticity response involving substrates other than glucose. Because oxidative fibers are less energetically demanding than glycolytic fibers (Lehnert et al., 2006), shifts toward a more oxidative muscle phenotype under nutritionally constrained conditions may represent a more efficient strategy for ATP production per unit of glucose (Ramírez-Zamudio et al., 2022). This metabolic reprogramming of muscle fibers is mediated, among other pathways, by AMPK, a cellular energy sensor (Rockl et al., 2007) that promotes the preferential utilization of fatty acids as an energy source (Merrill et al., 1997). Accordingly, the enrichment of this pathway in F1 LG heifers compared with the MG group (Figure 4) is compatible with such an adaptive metabolic profile. Because skeletal muscle acquires most of its fiber complement during gestation, transcriptomic alterations induced by early nutritional conditions likely reflect foundational shifts in metabolic development. Although direct phenotypic measurements of muscle were not available in this study, the molecular signatures observed here may be associated with long-term adjustments in metabolic regulation that support muscle development and function.

## 4.2 Impact of initial gestational rate of body weight gain in F0 dams on immune-associated transcriptomic regulation in the skeletal muscle of F1 offspring

In mammals, the development of the immune system begins in embryonic life and continues to mature after birth (Holsapple et al., 2003; Cortese, 2009). In ruminants, the immune system is primarily established during fetal development, whereas effective immune protection after birth depends on the passive transfer of immunoglobulins through colostrum intake and the neonate's absorptive capacity (Castro et al., 2005; Cardoso et al., 2021). Therefore, the maternal environment during gestation can have lasting effects on immune competence, with implications for animal health, welfare, and productivity (Cooke, 2019). Failures in this process are associated with increased susceptibility to disease, higher neonatal mortality, and reduced productive performance (Cardoso et al., 2021). In this context, the maternal environment plays a central role in determining offspring health, with direct implications for animal welfare, sanitary management, and the efficiency of production systems (Cooke, 2019).

Several biological processes related to immunity, such as leukocyte migration, antigen presentation, and inflammatory responses, were upregulated in F1 offspring from MG dams compared with those from the LG group. These findings indicate that maternal nutrition in early gestation can modulate, at the molecular level, pathways associated with immunological processes in skeletal muscle, without allowing direct inference of changes in the offspring's systemic immune capacity. Nevertheless, the processes and genes regulated in muscle are also associated with events of muscle repair and myogenic differentiation (Tidball, 2017).

Among the genes highlighted within the immune-related enriched processes in F1 MG heifers, *ITGB2*, which was positively regulated relative to the LG group, plays a key role in cell adhesion and leukocyte migration. *TNFAIP3*, also positively regulated in the MG group, plays a role in inflammation regulation and protection against programmed cell death. These genes are also involved in pathways regulating muscle development via AKT signaling (Wu et al., 2017). The inhibition of the NF- $\kappa$ B-mediated apoptotic pathway by *ITGB2* and *TNFAIP3* reinforces their dual roles in local immune modulation and muscle development (Wu et al., 2017; Li and Xie, 2022). Studies in mouse cardiac muscle have shown that *TNFAIP3* expression is regulated by *miR-1*, a miRNA associated with both myogenesis and apoptosis (Li and Xie, 2022). As discussed previously, the downregulation of *miR-1* in F1 LG heifers at birth, compared to the upregulation in F1 MG heifers, suggests that in the latter group, muscle tissue is at a more advanced stage of myogenic differentiation.

The genes *BOLA-NC1* and *C4A*, involved in antigen presentation, opsonization, and pathogen elimination (Thompson-Crispi et al., 2014), were also upregulated in F1 MG heifers compared to the LG group. These genes belong to the major histocompatibility complex pathway, and their antigens have been

identified on the surface of regenerating muscle fibers (Honda and RoStami, 1989; Thompson-Crispi et al., 2014; Ribeiro et al., 2022). Regulation of the *TYROBP* gene, essential for immune signaling in NK cells and macrophages, together with *CORO1A*, associated with cell motility and phagocytosis, indicates greater activation of molecular pathways related to local immune signaling in skeletal muscle (Martin et al., 2006; Msheik et al., 2019; Lee et al., 2022). These transcriptomic modifications seem to reflect adjustments in the local inflammatory microenvironment, possibly linked to muscle differentiation and remodeling, rather than an increase in the systemic immune response.

## 4.3 Impact of F0 dam rate of body weight gain during early gestation on gene regulation and biological processes and metabolic pathways in F2 offspring fetuses

Multigenerational programming effects are attributed to various complex mechanisms, resulting in alterations in the development of offspring tissues and organs (Aiken et al., 2016; Caton et al., 2020). Herein, common biological processes and metabolic pathways were identified between the F1 offspring and their F2 fetuses affected by the maternal rate of body weight gain of F0 dams during the first trimester of gestation. In the F1 offspring at birth and F2 fetuses at 84 dpc from the MG group, NADH dehydrogenase complex assembly and thermogenesis processes were negatively regulated. In contrast, cell adhesion molecules (CAMs) were positively regulated. At the harvest of F1 offspring and their F2 fetuses, two common pathways and one biological process were identified: ribosome was negatively regulated in the MG group in both sets, in contrast to glycosylphosphatidylinositol (GPI)-anchor biosynthesis and liposaccharide metabolic process, which were positively regulated. Interestingly, the oxidative phosphorylation pathway was negatively regulated in the MG treatment in both F1 heifers at birth and harvest and in F2 fetuses.

Regarding gene regulation in the F1 and F2 offspring, the genes *TNFAIP3*, *ITGB2*, *EOGT*, *BOLA-NC1*, and *C4A* were highlighted as upregulated in F1 heifers at birth in the MG treatment compared to the LG group. These genes are associated with inflammatory response, cell adhesion, post-translational protein modification, and antigen presentation, suggesting enhanced local immune-related mechanisms and preservation of skeletal muscle structural integrity in the F1 generation. Enrichment of the CAM pathway, both in F1 heifers at birth and in F2 fetuses from the MG group, highlighted the genes *ITGB2* and *BOLA-NC1*, which mediate cell-cell and cell-matrix interactions and promote anchorage to the extracellular matrix. CAMs play a central role in structural stabilization, cytoskeletal organization, and migratory cellular behavior in differentiating calcium-dependent tissues (Zhang et al., 2011). Taken together, these findings suggest that maternal nutrition during early gestation may be associated, in F1 and F2 progeny from the MG group, with a more accelerated structural maturation and earlier organization of the skeletal muscle

extracellular matrix, with effects potentially maintained across generations.

The transmembrane protein (TMEM) family comprises numerous proteins whose functions remain largely uncharacterized. In skeletal muscle, only a limited number of *TMEM* proteins have been associated with direct regulation of muscle physiology (Luo et al., 2021). In the present study, the genes *TMEM150B* and *TMEM81* were positively regulated in F1 heifers from the LG group at harvest and in their F2 fetuses relative to the MG group, except for *TMEM81*, which was negatively regulated in F1 heifers from the LG group at harvest. *TMEM150B* acts as a negative regulator of BMP signaling (Keum et al., 2023), a pathway involved in *Pax7*<sup>+</sup> satellite cell proliferation and muscle regeneration (Wang et al., 2010). In an integrated manner, it had previously been suggested that the positive regulation of *bta-miR-133a-1* in F1 heifers from the LG group at harvest is associated with phases of myogenic differentiation in satellite cells, favoring postnatal muscle hypertrophy. In F2 fetuses, the positive regulation of *bta-miR-133a-1* in the LG group compared with the MG group may suggest an anticipation of myogenic differentiation, associated with the formation of a lower number of primary muscle fibers, since proliferative processes occur predominantly during the first half of gestation in cattle (Santos et al., 2022). In this context, the recurrent presence of *miR-133* in the F1 and F2 generations can be interpreted in light of the interactions between DNA methylation and post-transcriptional regulation mediated by miRNAs. These mechanisms act in a complementary manner to fine-control gene expression during myogenesis, with promoter methylation modulating transcription and miRNAs, including *miR-1* and *miR-133*, participating in the coordinated silencing of shared targets (Su et al., 2011). Integrative studies of the methylome and miRNAome in bovine skeletal muscle have identified these miRNAs as central components of epigenetic networks involved in muscle development (Huang et al., 2014). Recent evidence further demonstrates that maternal nutrition can modulate these epigenetic mechanisms in developing tissues, as observed in models of maternal overnutrition (Shokrollahi et al., 2025). Thus, the consistent pattern of *miR-133* regulation across the F1 and F2 generations supports its involvement in fundamental epigenetic frameworks underlying the multigenerational programming of myogenesis.

## 5 Conclusion

Periconceptual maternal nutrition, characterized by different rates of body weight gain, modulated gene regulation, biological processes, and metabolic pathways in F1 skeletal muscle, with effects observed at birth, during postnatal development, and extending to the F2 generation. In F1 offspring, moderate maternal weight gain during early gestation was associated with the regulation of genes and pathways linked to cell adhesion, local immune-related mechanisms, and structural maintenance of

skeletal muscle, with evidence of persistence across generations. Conversely, low maternal weight gain during early gestation was associated in F1 offspring with transcriptional adjustments in energy metabolism, amino acid biosynthesis, and myogenic dynamic related genes, consistent with metabolic adaptations that may support postnatal muscle growth. The recurrent regulation of *bta-miR-133a-1* in both F1 and F2 progeny from dams with low early gestational weight gain further suggests the involvement of epigenetic mechanisms in the coordination of these responses and warrant further investigation. Although this study is based on transcriptomic data, which provides an integrated view of regulatory activity in developing muscle, complementary approaches will be necessary to determine how these molecular patterns translate into physiological adjustments across generations.

## Data availability statement

All relevant data are within the paper and its Supplementary Information files. All RNA-sequencing data is publicly available on NCBI's Gene Expression Omnibus through GEO Series accession number GSE315783.

## Ethics statement

The animal study was approved by North Dakota State University Institutional Animal Care and Use Committee (IACUC #A19062 and #A20047. The study was conducted in accordance with the local legislation and institutional requirements.

## Author contributions

GDR-Z: Validation, Formal analysis, Data curation, Writing – original draft, Writing – review & editing, Visualization. WD: Supervision, Methodology, Conceptualization, Software, Validation, Visualization, Investigation, Data curation, Writing – review & editing, Formal analysis. FB: Investigation, Writing – review & editing, Methodology. AM: Investigation, Funding acquisition, Conceptualization, Writing – review & editing, Methodology. JH: Writing – review & editing, Investigation. KB: Investigation, Writing – review & editing. SU: Writing – review & editing, Investigation. KM: Writing – review & editing, Investigation. LR: Investigation, Writing – review & editing. AW: Methodology, Conceptualization, Funding acquisition, Writing – review & editing. PB: Investigation, Writing – review & editing. KS: Resources, Methodology, Writing – review & editing. JC: Project administration, Writing – review & editing, Methodology, Funding acquisition, Investigation, Conceptualization, Resources. CD: Investigation, Resources, Funding acquisition, Writing – review & editing, Methodology, Supervision, Conceptualization, Project administration.

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## Conflict of interest

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## Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fanim.2025.1725223/full#supplementary-material>

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