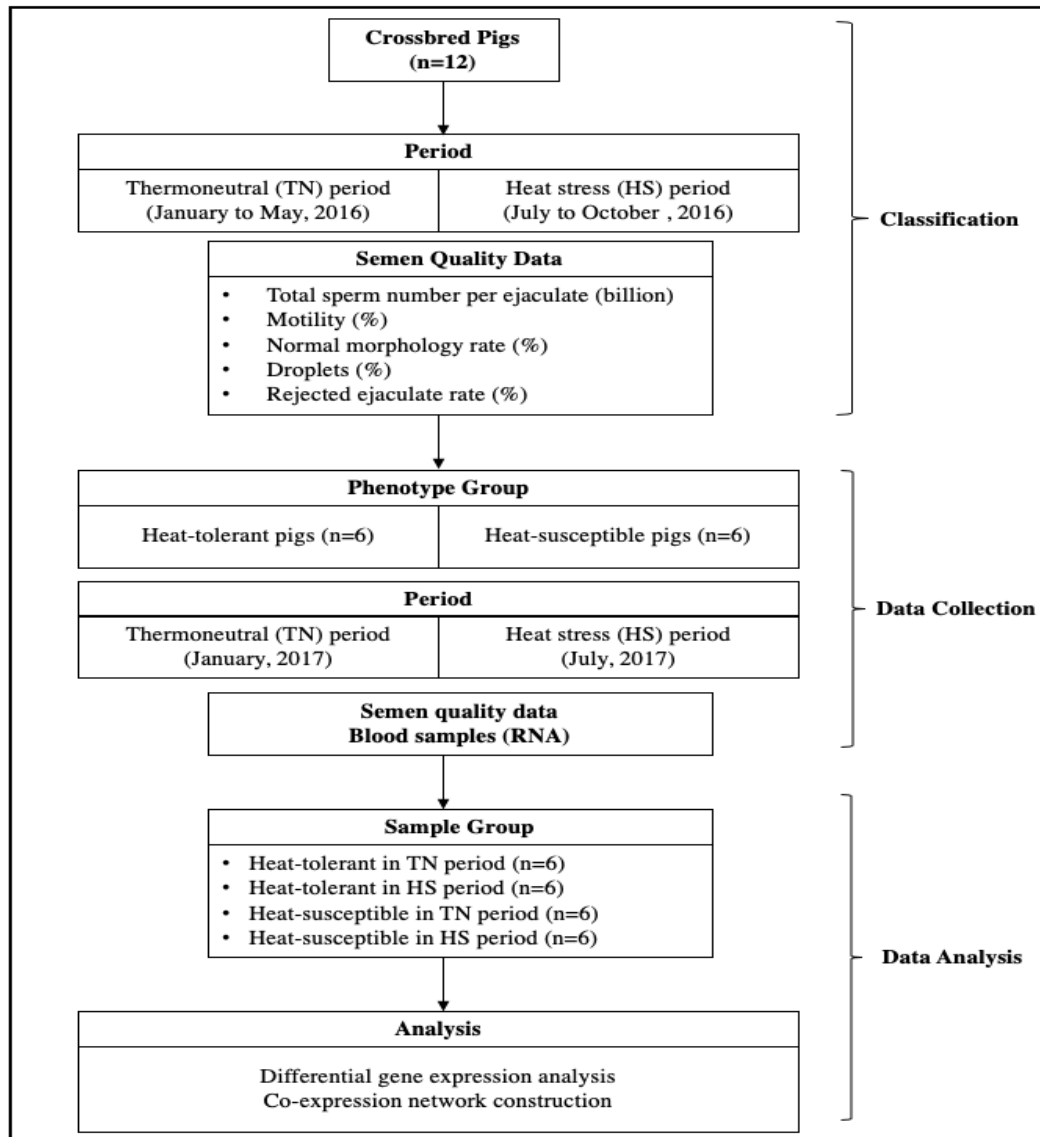


A



B

RNA-seq Analysis Pipeline	
<p>Processes:</p> <ul style="list-style-type: none"> • QC of Raw reads(.fastq) • Read Alignment (.bam) • Raw gene counts (.txt) 	<p>Tools:</p> <ul style="list-style-type: none"> • FastQC (v.0.10.1) • TopHat2 (v.2.0.14) • featureCounts (v.1.6.3)
Differential Gene Expression Analysis (edgeR and Limma)	Co-expression Network Analysis (edgeR and WGCNA)
<p>Data Processing:</p> <ul style="list-style-type: none"> • Raw count (genes = 25880) • Remove low count (genes = 10761) • Normalization using Trimmed Means method (TMM) • Transformed to counts per million (CPM) <p>Data Analysis:</p> <ul style="list-style-type: none"> • To_HS vs. To_TN • Su_HS vs. Su_TN • To_HS vs. Su_HS • To_TN vs. Su_TN <p>Gene Annotation:</p> <ul style="list-style-type: none"> • KEGG pathway mapping • GO term enrichment analysis 	<p>Data Processing:</p> <ul style="list-style-type: none"> • Raw count (genes = 25880) • Remove low count (genes = 10761) • Normalization using Trimmed Means method (TMM) • Transformed to reads per kilobase of transcripts per million-mapped (RPKM) • Transformed to log2 <p>Data Analysis:</p> <ul style="list-style-type: none"> • Module detection within To_HS • Module detection within Su_HS • Correlation with semen quality parameters <p>Gene Annotation:</p> <ul style="list-style-type: none"> • KEGG pathway mapping • GO term enrichment analysis
Results	
<ul style="list-style-type: none"> • Differentially expressed genes (DEGs) • Network modules with hub genes • Relationships between modules and semen quality parameters • Functional enrichment terms of DEGs and genes within modules • KEGG pathways associated with DEGs and genes within modules 	