

# Dkk3/REIC Deficiency

Subjects: [Andrology](#)

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The role of Dickkopf-3 (Dkk3)/REIC (The Reduced Expression in Immortalized Cells), a Wnt-signaling inhibitor, in male reproductive physiology remains unknown thus far.

Dkk3/REIC

fibrous sheath

knock-out

RNA-seq

## 1. Introduction

Infertility is a serious problem that affects over 14% of couples in the world, and half of the cases can be attributed to male factors [1]. With the help of modern techniques in molecular biology, genetic disorders have been proven to be a major cause of male infertility, especially for those with non-obstruction in the genital ducts [2]. To date, over 2000 genes have been reported to participate in male reproductive physiology, and new findings continue emerging [3]. According to recent studies, Wnt signaling, a classical intercellular pathway that regulates cellular proliferation and tissue development, plays a pivotal role in male reproductive function [4][5].

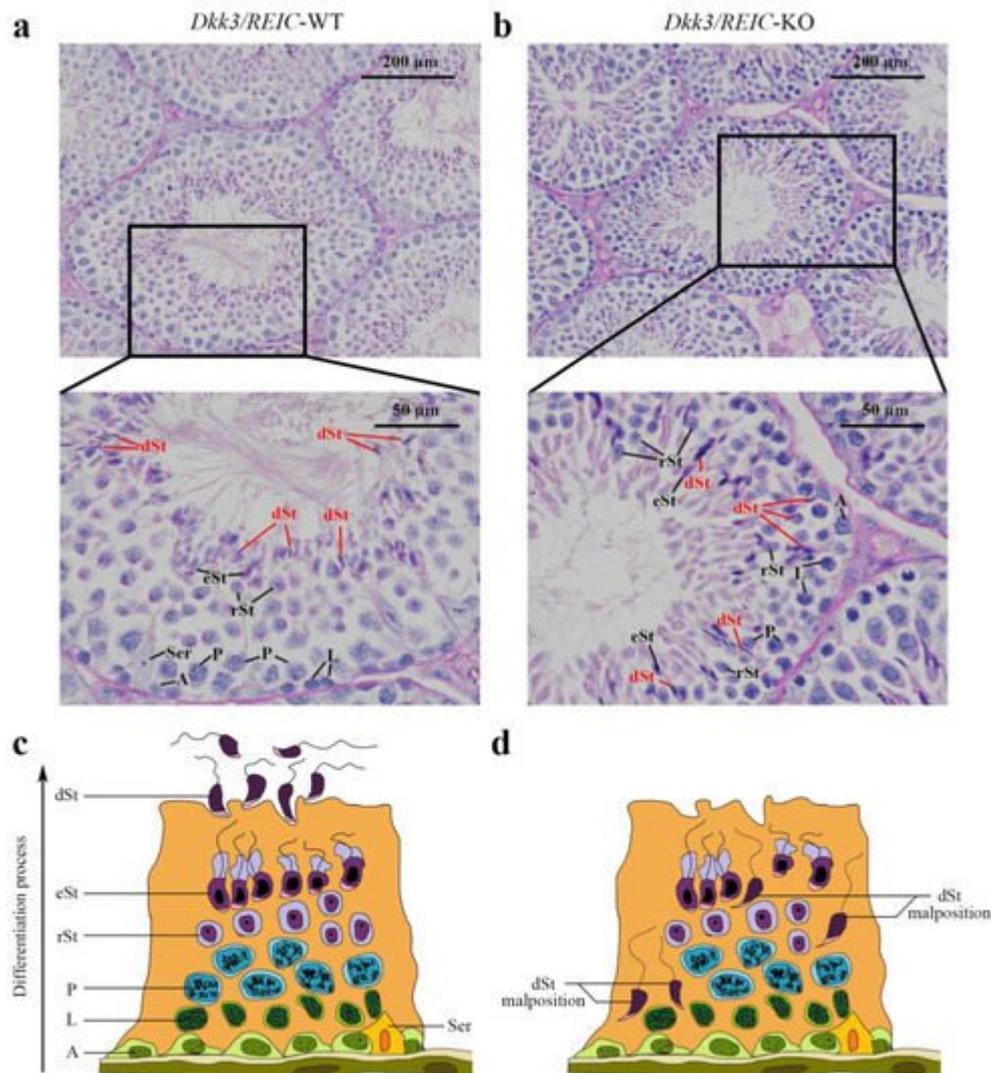
Among the Wnt signal community, *Dickkopf* (*Dkk*) family, a negative regulator of Wnt signaling, has recently attracted attention from scholars in male reproduction fields. Currently, five members of the family have been found, which are known as *Dkk1-4* and *Dkk11* (*Dickkopf-like protein 1*) [6]. For years, their institution has been dedicated to the research of *Dkk3* and first identified its human homologous counterpart as a gene whose expression is reduced in immortalized cells, thus, also named *Dkk3* as *REIC* (*The Reduced Expression in Immortalized Cells*) [7].

*Dkk3/REIC* shares high sequence similarity with *Dkk11*, and they are believed to arise from the same ancestral precursor [6]. There are several studies reporting that *Dkk11* is deeply involved in the male reproductive process, as overexpressing *Dkk11* in male mice can severely impair their spermatogenesis, and sperm from *Dkk11*<sup>-/-</sup> mice exhibit a decreased sperm-egg binding capacity [8][9].

## 2. Spermiation Failure and Malposition of Differentiated Spermatids Exist in Testicular Sections of Dkk3/REIC-KO Mice

To investigate morphological changes of spermatogenesis after *Dkk3/REIC* was knocked out, the PAS-stained testicular sections of *Dkk3/REIC*-KO and *Dkk3/REIC*-WT mouse were examined. Researchers noticed that spermiation failure existed in *Dkk3/REIC*-KO mice testes, and disordered placement of differentiated spermatids

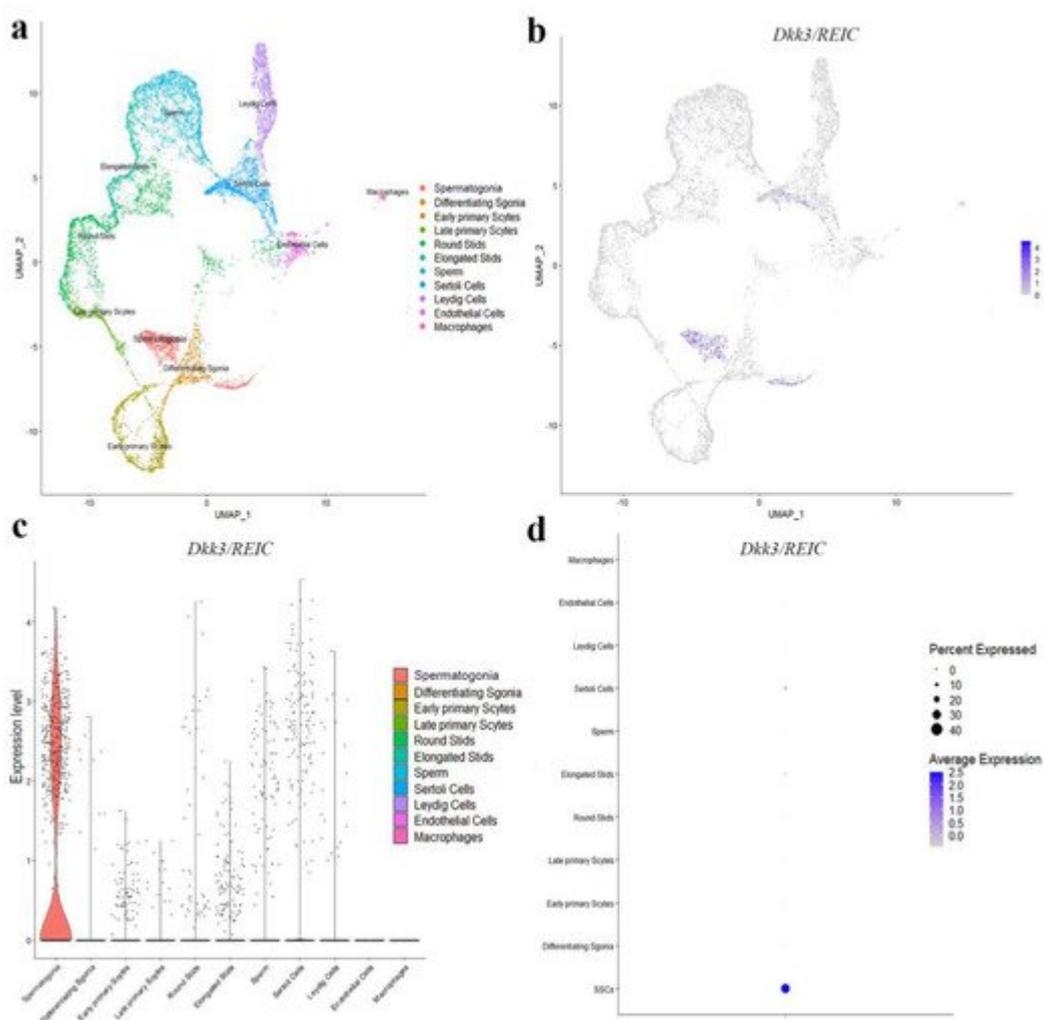
(which was most clearly displayed in stage VIII–IX) was commonly found in their testicular seminiferous tubules. As shown in **Figure 1**, differentiated spermatids were absent from the centers of the tubules and irregularly wandered adjacent to the basement membrane. However, the average amount of testicular germ cells revealed no significant difference between the *Dkk3/REIC*-WT and *Dkk3/REIC*-KO mice.



**Figure 1.** PAS staining of the testicular sections. (a) In the *Dkk3/REIC*-WT testicular section (stage VIII–IX of spermatogenesis), the distribution of germ cells was well arranged, and the tadpole-like differentiated sperm were uniformly located around the seminiferous tubule lumen waiting to be released. (b) In the *Dkk3/REIC*-KO testicular section (stage VIII–IX of spermatogenesis), however, the germ cell distribution was much disordered, few mature spermatids were in or surrounding the central lumen, while the differentiated sperm (marked by in red) irregularly wandered adjacent to the basement membrane. (c) The schematic drawing of typical spermatogenesis in the *Dkk3/REIC*-WT seminiferous epithelium. (d) The schematic drawing of pathological spermatogenesis in the *Dkk3/REIC*-KO seminiferous epithelium. Abbreviation: A = spermatogonia, Ser = sertoli cell, L = spermatocytes in leptotene phase, P = spermatocytes in pachytene phase, rSt = round spermatids, eSt = elongated spermatids and dSt = differentiated spermatids.

### 3. Expression and Distribution of *Dkk3/REIC* in Testes and Sperm of Male Mouse

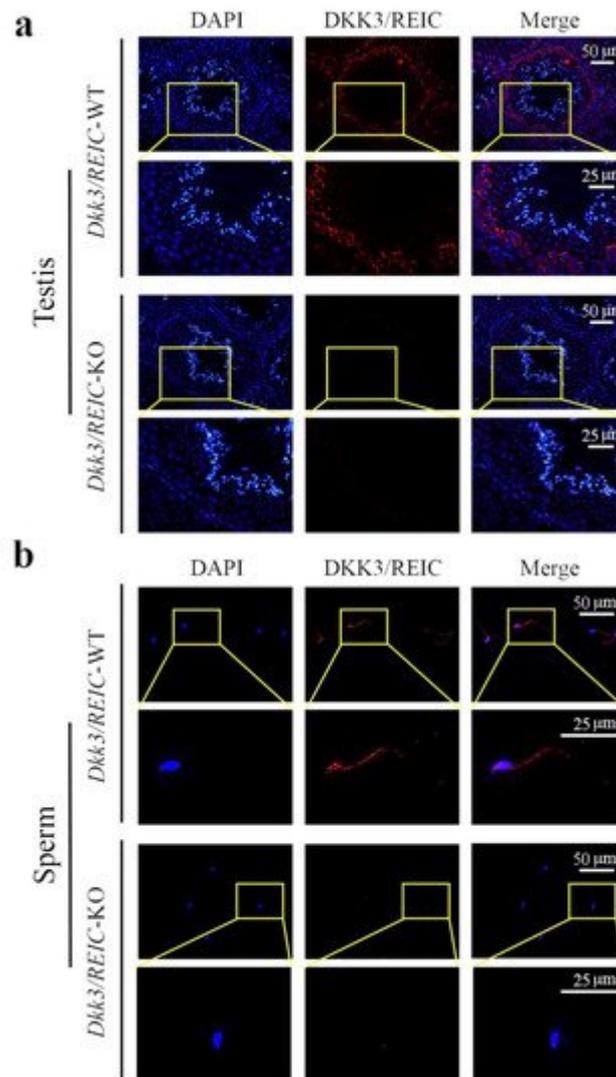
To explore the transcript expression of *Dkk3/REIC* in mouse testes, they used the published C57BL/6 testicular scRNA-seq transcriptome in GEO database (GSE112393) and assessed the cell-specific expression of *Dkk3/REIC*. Clustering analysis identified 11 types of testicular cells (**Figure 2a**), *Dkk3/REIC* transcripts existed in most of the cell types except for macrophages and endothelial cells (**Figure 2b,c**), and this was predominately expressed in spermatogonia cells (**Figure 2d**). Then, researchers used testicular sections and sperm smear obtained from KO and WT mice and detected *Dkk3/REIC* expression by immunofluorescence (IF) staining.



**Figure 2.** Distribution of *Dkk3/REIC* among testicular cell clusters in public scRNA-seq transcriptome atlas. (a) Analysis revealed 11 clusters that were matched to 11 testicular cell types. (b) The FeaturePlot function, (c) VlnPlot function and (d) DotPlot function of Seurat packages demonstrated the distribution of *Dkk3/REIC* among these different cell types. The results indicated that *Dkk3/REIC* had the highest level in spermatogonia.

As shown in **Figure 3a**, *DKK3/REIC* in the testicular section was mainly expressed in spermatogonia. This was compliant with their results from the published scRNA-seq data. **Figure 3b** shows the distribution of *DKK3/REIC* in

mouse sperm, which was extensively expressed in both the head and tail, indicating that it may be a functional protein for spermatic activity.



**Figure 3.** Expression and localization of DKK3/REIC protein in mouse seminiferous tubules and sperm. The expression and distribution of DKK3/REIC protein in (a) testes and (b) sperm of *Dkk3/REIC*-WT and *Dkk3/REIC*-KO mice.

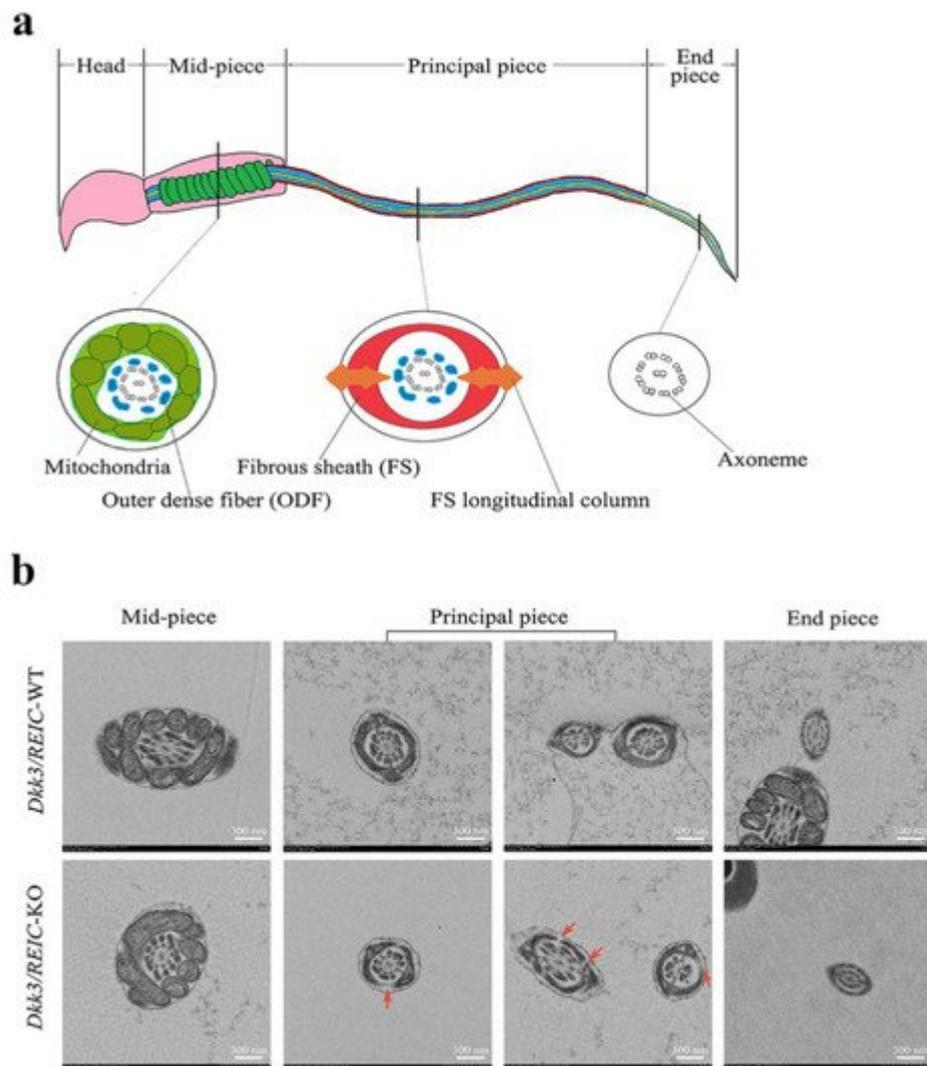
## 4. Sperm Motility Is Impaired in *Dkk3/REIC*-KO Male Mice While the Sperm Total Count and Vitality Rate Show No Significant Difference

Although the average epididymal sperm count (per epididymis) of *Dkk3/REIC*-KO mice was nearly 1 million less than their wild-type counterparts ( $9.30 \pm 0.69$  vs.  $8.27 \pm 0.87$ ,  $\times 10^6$ ), the difference was not statistically significant ( $p > 0.05$ ). Sperm vitality rates between the WT and KO mice were also non-significant ( $72.83 \pm 1.55\%$  vs.  $72.50 \pm 0.71\%$ ,  $p > 0.05$ ). Irregular findings were noticed in the motility status of *Dkk3/REIC*-KO mice sperm. Compared

with the wild-types, KO mice showed a significant lower proportion of motile sperm ( $44.09 \pm 8.12\%$  vs.  $23.26 \pm 10.02\%$ ,  $p < 0.01$ ).

## 5. Ultrastructural Examination Reveals Defects in Flagellum Fibrous Sheath of *Dkk3/REIC*-KO Mouse Sperm

The normal sperm structure under TEM is depicted in **Figure 4a**. Sperm flagellum can be routinely divided into three sub-parts, which are the mid-piece, principal piece and end piece [10]. The cross sections of different sub-parts contain different structures. For *Dkk3/REIC*-KO sperm, irregular changes were found in the fibrous sheath, a major component of the principal piece. Compared with their wild-type counterparts, which showed an intact fibrous sheath, *Dkk3/REIC*-KO sperm had multiple defects and fractures in the fibrous sheath of the flagellum (**Figure 4b**). Such abnormalities could disrupt the structural continuity of the sperm flagellum, which might be linked with the sperm motility impairment in *Dkk3/REIC*-KO mice.

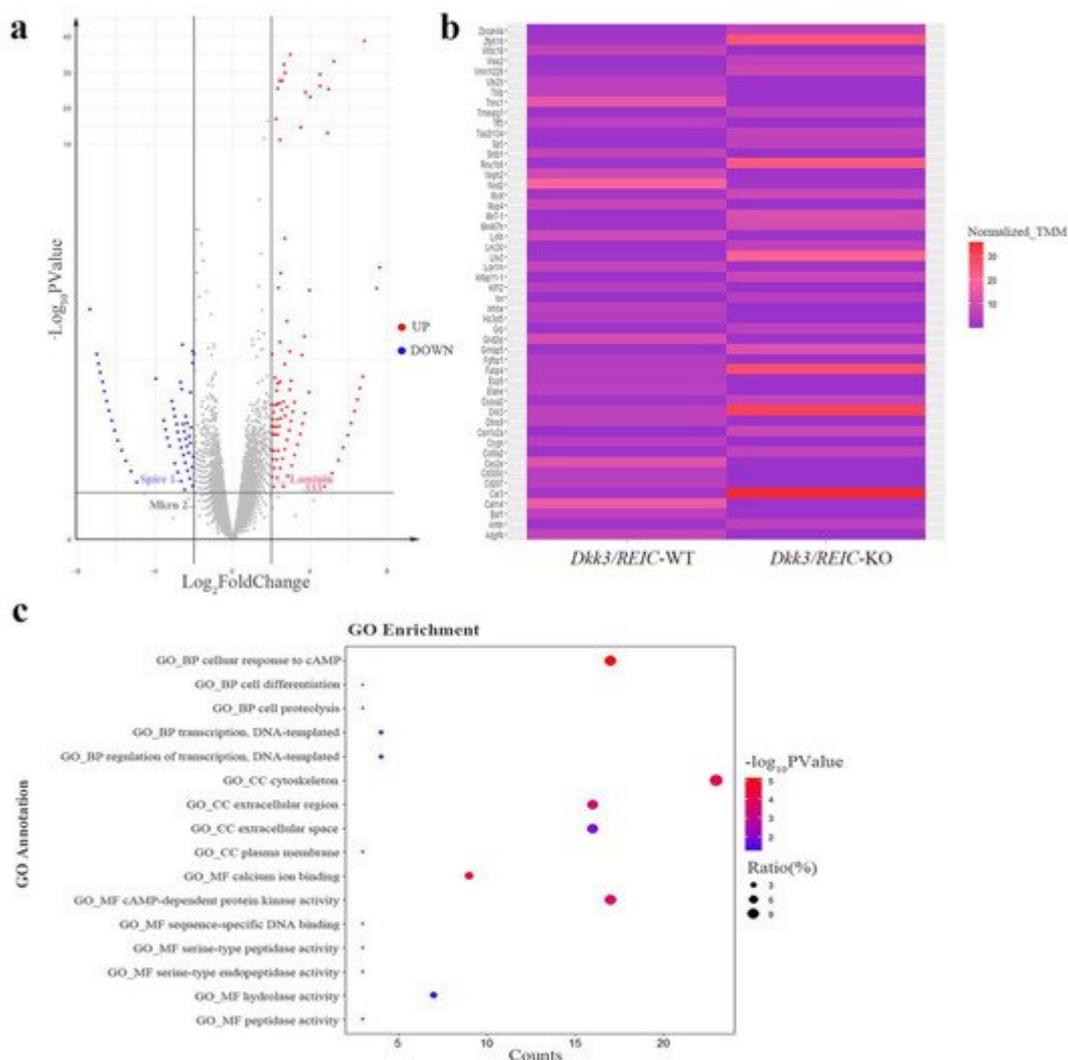


**Figure 4.** Transmission electron microscope observation of *Dkk3/REIC*-WT and *Dkk3/REIC*-KO sperm. (a) Schematic drawing of the mouse sperm ultrastructure. The sperm flagellum can be divided into three parts, which

are the mid-piece, principal piece, and end piece. Their cross section views are different from each other, and the fibrous sheath structure mainly exists in the principal piece. (b) The TEM images of *Dkk3/REIC*-WT and *Dkk3/REIC*-KO sperm. No obvious difference was found between the mid-piece and end piece; however, the principal piece of *Dkk3/REIC*-KO sperm showed irregular fractures in the fibrous sheath (arrows).

## 6. RNA Sequencing of Testicular Transcriptome from *Dkk3/REIC*-WT and *Dkk3/REIC*-KO Mice and GO Enrichment Analysis

The RNA-seq of testes transcriptome revealed 36,060 DEGs. The DEGs with FDR value less than 0.05 and a fold change larger than 2 are marked by colors in the volcano plot (Figure 5a). The relative expressions of top 50 DEGs between *Dkk3/REIC*-WT and *Dkk3/REIC*-KO testes are shown in a heatmap (Figure 5b). Then, researchers selected the top 200 DEGs as candidate genes and conducted GO (Gene Ontology) enrichment analysis. As shown in Figure 5c, for the GO category of Biological Process (BP), the candidate genes were predominately enriched in the *cellular response to cAMP*.



**Figure 5.** Differentially expressed genes and enrichment results of *Dkk3/REIC*-WT and *Dkk3/REIC*-KO mice. **(a)** Volcano plot of differentially expressed genes (DEGs). Genes with significant difference (FDR < 0.05 and fold change  $\geq 2$ ) are marked in colors. Red and blue, respectively, indicate the up-regulated and down-regulated genes in *Dkk3/REIC*-KO mice. DEGs of *Laminin-333*, *Spire 1* and *Mkrn 2* are marked by labels. **(b)** The relative expressions of the top 50 DEGs among *Dkk3/REIC*-WT and *Dkk3/REIC*-KO mice testes. **(c)** The top 200 DEGs analyzed by Gene Ontology (GO) enrichment.

In the Cellular Component (CC) category, candidates were significantly concentrated in the *cytoskeleton*. For the Molecular Function (MF) category, *calcium ion binding* and *cAMP-dependent protein kinase activity* were the most significantly enriched GO terms. Collectively, the GO enrichment results indicated that the functions of the selected DEGs were mainly focused on cAMP signaling, calcium ion binding and cytoskeleton function, revealing that the absence of *Dkk3/REIC* may induce changes in the above activities and consequently lead to reproductive impairment.

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