Abstract: Hyperlipidemia inhibits bone formation, which has an adverse impact on the success of dental implants. Fzd9 has been reported to be a positive regulator of osteogenesis. However, the role of Fzd9 in dental implants in hyperlipidemic conditions is still unknown. In current study, we investigated how Fzd9 impacts on implant osseointegration in hyperlipidemic conditions. Models of high-fat medium-stimulated rat bone marrow stromal cells (BMSCs) were established. Alkaline phosphatase (ALP), alizarin red S (ARS) and oil red O (ORO) staining were used to examine the osteogenic differentiation of BMSCs. Wistar rats were fed with high-fat diet to induced hyperlipidemia. Titanium implants were implanted into the proximal metaphysis of the bilateral femurs of rats after 8 weeks. Thereafter, 1 mm of bone around each implant was obtained. Implant osseointegration and micromorphology were analyzed with microcomputer tomography (micro-CT) and hematoxylin-eosin (HE) staining. The relative expression levels of Fzd9 and Runx2 were analyzed by quantitative real-time PCR in vitro and in vivo. Western blotting was used to analyzed the protein level of Fzd9. The expression levels of Fzd9 and Runx2 were decreased. Osteogenic differentiation of BMSCs were suppressed under high-fat medium. Less bone formation was observed in hyperlipidemic rats compared to the normal. Hyperlipidemic rats had lower osteoblasts and bone-implant combination (BIC), bone volume fraction (BV/TV), trabecular thickness (Tb.Th), trabecular number (Tb.N) but higher trabecular spacing (Th.S), trabecular bone pattern factor (TBF) and osteoclasts. In conclusion, the lower expression of Fzd9 impairs osseointegration in hyperlipidemic conditions via Wnt signaling pathway-related Runx2. Fzd9 may serve as a promising strategy for hyperlipidemia osseointegration.

Key words: BMSCs, Frizzled-9, Hyperlipidemic rats, Implant osseointegration

Introduction

It is well known that dental implants serve as a common prosthetic method for partially or completely edentulous patients, and the long-term success rate depends on successful implant osseointegration. Hyperlipidemia is a metabolic disease characterized by the elevation of serum lipids derived from a high-fat (HF) diet or atherosclerosis. Recently, cumulative evidence has confirmed that hyperlipidemia reduces bone formation and results in considerable bone loss in mice. Some studies suggested that the mouse bone marrow microenvironment was altered by HF diet feeding with an increase in osteoclast precursors as well as increased expression of the osteoclastogenic regulator peroxisome proliferator-activated receptor-gamma (PPARγ), further indicating that PPARγ stimulates adipogenesis and inhibits osteogenesis, which may impair osteoblast differentiation and result in decreased bone formation. Furthermore, our previous studies have all elucidated that mice fed a HF diet had significantly less bone formation and reduced strength of the bone-to-implant interface as well as increased implant loss. Based on the adverse influence of hyperlipidemia on bone metabolism, it is important to investigate the mechanism of hyperlipidemia on implant osseointegration.

The Wingless/int (Wnt) signaling pathway has become a hot research topic in bone biology for more than a decade and can be activated in canonical/β-catenin and noncanonical pathways by Wnt ligands. In the canonical/β-catenin signaling pathway, extracellular Wnt ligands bind to the low-density lipoprotein receptor-related protein (LRP5)-Frizzled receptor (Fzds) complex and then activate intracellular disheveled (DSH). Subsequently, the intracellular complex of axin, glycogen synthase kinase3 (GSK3), and adenomatosis polyposis coli (APC) protein were repressed, which suppressed the phosphorylation of β-catenin. This process causes β-catenin to accumulate and then enter the nucleus, where it mediates the transcription of Wnt genes, such as Runx2, the master regulator of osteogenesis, to regulate bone metabolism. The noncanonical signaling pathway, which is independent of β-catenin, is also activated by Fzds binding to the extracellular Wnt. Thus, Fzds play a key role in initiating the Wnt signaling pathway. Canonical and noncanonical Wnt signaling pathways exert mutual functions at the cell surface by competitively binding to Fzds. Fzds are seven transmembrane cell surface receptors and include Fzd1-10. They have a wide range of regulatory functions not only in initiation and reduced strength of the bone-to-implant interface as well as increased implant loss. Based on the adverse influence of hyperlipidemia on bone metabolism, it is important to investigate the mechanism of hyperlipidemia on implant osseointegration.
tion of the Wnt signaling pathway to modulate bone metabolism, but also in embryo development in the process of stem cell differentiation and maintaining tissue regeneration and restoration. Of all the Fzd family members, Fzd9 is a major focus of bone research, which is the only result of Fzd9-deficient mice displaying low bone mass formation mainly via noncanonical Wnt signaling pathways. Therefore, we speculate that Fzd9 may also play a significant role in hyperlipidemic rat osteogenesis.

Based on the adverse influence of hyperlipidemia on bone metabolism and the importance of Fzd9 in the Wnt signaling pathway for bone remodeling, studying Fzd9 might provide insights into the potential molecular mechanism of implant osseointegration in hyperlipidemic conditions.

Materials and Methods
Preparation of basal osteogenic induction medium and HF osteogenic induction medium
Dexamethasone (Solarbio Science and Technology Co., Ltd., Beijing, China) 0.00393 g was weighed out in the dark and dissolved in 2 ml anhydrous ethanol. Then, 3 ml 10% FBS (Thermo Fisher Scientific, MA, USA) α-MEM (Hyclone Laboratories Inc., Logan, Utah, USA) medium was added and filtered with a 0.22 µm filter and then diluted with 10% FBS α-MEM medium. Meanwhile, 0.306 g sodium β-glycerophosphate with 5H2O (Solarbio Science and Technology Co., Ltd., Beijing, China) and 0.005 g vitamin C (Solarbio Science and Technology Co., Ltd., Beijing, China) were dissolved in 5 ml 10% FBS α-MEM medium and filtered with a 0.22 µm filter. Then, 2.5 ml of solution was added to the above 47.5 ml of 10% FBS α-MEM medium. Repeating the process with a HF medium (PythonBio Laboratories Inc., Guangzhou, China), HF osteogenic induction medium can be obtained.

BMSC culture, ALP, ARS and ORO staining
Bone marrow stromal stem cells (BMSCs) were taken from 3-week-old Wistar male rats and cultured to the third generation. Then, BMSCs were randomly divided into experimental and control groups. Experimental groups were cultured in HF osteogenic induction medium and control groups with basal osteogenic induction medium. After 7, 21 and 28 days of osteogenic induction, BMSCs were stained with ALP (Beyotime Biotechnology Co., Ltd., Shanghai, China), 0.2% Alizarin Red S (ARS) (Solarbio Science and Technology Co., Ltd., Beijing, China) and oil red O (ORO) (Solarbio Science and Technology Co., Ltd., Beijing, China) according to the manufacturer’s instructions. An inverted microscope was used to observe and photograph the cells.

ALP, ARS and ORO staining and quantification analysis
The alkaline phosphatase (ALP) activity was quantified by the ALP Colorimetric assay kit (Jiancheng Biotechnology Co., Ltd., Nanjing, China) according to the manufacturer’s instructions. Protein concentrations were detected by Enhanced BCA protein assay kit (Solarbio Science and Technology Co., Ltd., Beijing, China).

For quantification of mineralization, the staining was dissolved by 100 mM hexadecylpyrriridinium chloride monohydrate (Solarbio Science and Technology Co., Ltd., Beijing, China) for 30 min at room temperature and measured by SPECTROstar Nano (BMG Labtech Ltd.) at 562 nm.

The red lipid drops were dissolved by isopropyl alcohol at room temperature for 30 min and detected by SPECTROstar Nano at 510 nm.

Real-time quantitative PCR of Fzd9 and Runx2 in BMSCs
After 5, 10, 15 and 20 days of osteogenic induction, BMSCs were extracted with TRizol reagent (TaKaRa Bio Inc., Kyoto, Japan) according to the manufacturer’s instructions. Specific primer sequences of Fzd9 and Runx2 were synthesized (BioSune Biotechnology Co., Ltd., Shanghai, China). The corresponding primers are listed in Table 1. Total RNA (1 µg) was reverse transcribed into complementary DNA (cDNA) with a cDNA synthesis kit (TaKaRa Bio Inc., Kyoto, Japan) according to the manufacturer’s guidelines. The reaction products of Fzd9 and Runx2 were amplified by RT-PCR with a LightCycler 480II/96 (Roche Diagnostics Ltd.) using a standard SYBR Green PCR kit (TaKaRa Bio Inc., Kyoto, Japan). The relative expression levels of target genes were normalized against GAPDH.

Western blot analysis of Fzd9 protein in BMSCs
At days 5, 10, 15 and 20 after osteogenic induction, BMSCs were lysed in RIPA (Solarbio Science and Technology Co., Ltd., Beijing, China) lysis buffer with PMSF (Boster Biological Technology Co., Ltd., Wuhan, China). A bicinchoninic acid (BCA) protein assay kit (Solarbio Science and Technology Co., Ltd., Beijing, China) was used to determine protein concentrations according to the standard curve. Equal amounts of proteins were analyzed by SDS–polyacrylamide gel electrophoresis (PAGE) (Boster Biological Technology Co., Ltd., Wuhan, China) and electrotransferred to PVDF membranes (Invitrogen, Carlsbad, CA, USA). Membranes were washed three times with Tris-buffered saline/Tween-20 (TBST) and blocked with 5% nonfat milk in Tris-buffered saline at room temperature for 2 h, then incubated with primary antibodies against Fzd9 (diluted 1:750, Proteintech, USA) overnight at 4°C and matched horse radish peroxidase (HRP)-conjugated secondary antibody (1:3000; Proteintech, USA) at room temperature for 2 h. GAPDH (diluted 1:10000, Proteintech, USA) was blotted on the same membrane as a loading control. Bands were visualized by the Lumi-Q HRP Substrate solution kit (Servicebio Technology Co., Ltd., Wuhan, China) and analyzed with the AlphaView SA system.

Animals
Seventy 4-week-old male Wistar rats were provided by Shandong...
University Experimental Animal Center (Ji’nan, China). All experimental protocols were guided and approved by the Institutional Animal Care and Use Committee of the School of Stomatology of Shandong University (Ji’nan, China) (Approval Number: GB201817). All measures were taken to minimize pain or discomfort.

The rats were randomly divided into hyperlipidemic and normal groups. Hyperlipidemic groups were fed a HF diet. The HF diet included basic feed (78.8%), lard (10%, w/v), egg yolk powder (10%, w/v), cholesterol (1%, w/v) and bile salt (0.2%, w/v), which was purchased from Beijing Ke’axieli Co., Ltd. (Beijing, China), and the control groups were fed a normal diet. After 8 weeks, blood from the endocardial vein was collected to detect blood lipids, including serum total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL) and low-density lipoprotein (LDL). TC in the hyperlipidemic groups was approximately 2-folds higher than that in the normal groups, and TG and LDL were significantly higher than those in the normal groups (P<0.05) (Table 2). The rats were considered hyperlipidemic rats. Hyperlipidemic rats were fed a HF diet during the whole experiment.

**Implantation and tissue preparation**

After blood detection, rats were anesthetized with 10% chloral hydrate (0.3 ml per 100 g of weight) by intraperitoneal injection. A 2 cm full thickness incision was cut on the anteromedial portion of the bilateral femurs. The implant site was prepared using a drill 1.3 mm in diameter by a surgical micromotor (800 rpm) under constant irrigation with 0.9% sterile physiological saline. Sterilized titanium root-form implants (1.5 mm in diameter, 2.5 mm in length) (Jiangsu Shuangyang Medical Instrument Co., Ltd., Suzhou, China) were inserted. Postoperatively, every rat was given penicillin 0.06 ml/kg intramuscularly daily for 3 days.

At days 5, 10, 15, 20 after implantation, rats from two groups were sacrificed under deep anesthesia with chloral hydrate. After 1 mm of bone tissue surrounding the dental implants was dissected, it was instantly frozen in liquid nitrogen and stored at -80°C.

**Microcomputer tomography (Micro-CT)**

At day 20 after implantation, the left samples from the two groups were fixed in 4% paraformaldehyde for 24 h and were scanned with Inveon MicroCT (Inveon Acquisition Workplace, Siemens Ltd., Munich, Germany) at a resolution of 9.08 microns. One millimeter of bone around the implants was selected as the region of interest (ROI) to analyze bone trabecular parameters in ROI, including bone volume fraction (trabecular bone volume/ROI volume, BV/TV), trabecular thickness (Tb. Th), trabecular number (Tb. N), trabecular spacing (Tb. S) and trabecular bone pattern factor (TbF). Then, three-dimensional reconstruction of the trabecular bone in the ROI was performed by COBRA_Exxim (EXXIM Computing Corp., Livermore, CA) to characterize the bone-implant interface.

**Hard tissue slices and staining**

After micro-CT scanning, the samples were dehydrated in ascending series of ethanol and embedded with resin. Then they were sectioned by a section cutter in the longitudinal direction parallel to the long axis of the implant. Approximately 30 μm thick sections were obtained with a grinding machine (E400CS, EXAKT Inc., Vertriebs GmbH, Germany) and a parallel adhesive tablet device (E401/402, EXAKT Inc., Vertriebs GmbH, Germany) to stain with hematoxylin-eosin (HE). Inverted microscopy was used to observe and photograph the interface of the bone implant. Image-Pro Plus software was used to measure the bone shape size of the bone-implant interface, the thread length and the length that implants were bonded directly to bone, and calculate the bone-implant combination (BIC) ratio. The number of osteoblasts and osteoclasts in each group were also calculated by Image-Pro Plus software.

**Real-time quantitative PCR of Fzd9 and Runx2 in bone tissue**

After implantation at 5, 10, 15, and 20 days, total RNA from the two groups was extracted from frozen tissue using FastPure Cell/Tissue Total RNA Isolation Kit (Vazyme Biotech Co., Ltd., Nanjing, China) according to the manufacturer’s instructions. The corresponding primers are listed in Table 1. The expression levels of Fzd9 and Runx2 were measured by RT-PCR, and the method was the same as that described above.

**Western blot analysis of Fzd9 protein in bone tissue**

Total proteins from the two groups were extracted from frozen tissue at 5, 10, 15, and 20 days using Minute³⁰ Total Protein Extraction Kit for Bone Tissue (Invent Biotechnologies, Inc., Eden Prairie, USA) according to the manufacturer’s instructions. 50 μg of proteins from each sample were loaded on SDS–polyacrylamide gel electrophoresis (PAGE) and electrotransferred to PVDF membranes (Invitrogen, Carlsbad, CA, USA). And the remaining methods were the same as that described above.

**Statistical analysis**

Data from the independent experiments were analyzed by GraphPad Prism 6.0 for Windows (GraphPad Software Inc., USA), and values were expressed as the mean ± SD. Comparisons were analyzed by using a t-test, and P<0.05 was considered statistically significant.

**Results**

**Hyperlipidemia inhibited the differentiation of BMSCs**

ALP, ARS and ORO staining and quantification analysis were used to detect the differentiation of BMSCs cultured in a basal and hyperlipidemic environment (Fig. 1).

ALP staining at day 7 showed fewer and shallower hyperchromatic osteoblasts in the experimental groups than in the control groups (Fig. 1a, b). ALP activity in the control groups increased 2.07-folds compared to the experimental groups (Fig. 1c, d). The quantification analysis (Fig. 1b) showed hyperlipidemia environment suppressed osteoblast differentiation. Given that the HF diet is apt to induce more adipogenesis and that a higher level of adipogenesis is correlated with a lower level of osteogenesis, we next examined the adipogenesis of cultured BMSCs in a hyperlipidemic environment.

| Table 2. The serum TC, TG, HDL and LDL of two groups (x±s mmol/l, n=35) |
|---|---|---|---|
| Control groups | 1.34±0.23 | 0.82±0.26 | 0.85±0.21 | 0.25±0.05 |
| Experimental groups | 2.85±1.34* | 1.73±0.66* | 1.07±0.21* | 0.44±0.16* |

*Significant differences between control groups and experimental groups (P<0.05).
Using ORO staining. After 28 days of osteogenesis induction, the experimental groups had more red oil drops than the control groups (Fig. 1e, f, i). These results illustrated that hyperlipidemia could impair osteogenesis and induce adipogenesis.

**RT-PCR results in BMSCs**

To investigate the potential molecular mechanisms of adverse osteogenesis, BMSCs were collected for RT-PCR analysis at 5, 10, 15, and 20 days (Fig. 2). The expression of Fzd9 in the experimental groups was lower than that in the control groups at days 5, 10, 15 (P< 0.05) (Fig. 2a), but there was no obvious difference at day 20 (P> 0.05) (Fig. 2a). Compared with the control groups, there was lower Runx2 expression in the experimental groups at days 5 and 15 (P< 0.05), but there was no obvious difference at days 10 and 20 (P>0.05) (Fig. 2b). These results showed that impaired osteogenesis might be regulated by the decreased expression of Fzd9.

**Western blot of Fzd9 in BMSCs**

To further confirm the expression of Fzd9 in BMSCs, the translation level of Fzd9 was examined by Western blot. The result (Fig. 3) was consistent with the RT-PCR results. The protein level of Fzd9 in the experimental groups was decreased than that in the control groups at days 5, 10, 15, and 20.

**Establishment of hyperlipidemic rats**

The TC level was increased approximately two-folds; TG, HDL and LDL levels were enhanced in the hyperlipidemic groups and were significantly higher than those of the control groups (Table 2).
Hyperlipidemic rats were successfully induced by a HF diet.

**Micromorphology of samples**

1. Three-dimensional reconstruction

Trabecular bone in the ROI was reconstructed on day 20 (Fig. 4a). More regular and denser trabecular bone was observed in the normal groups than in the hyperlipidemic groups.

2. Histomorphometric quantification of trabecular bone in the ROI

   Figure 3. Western blot of Fzd9 proteins in BMSCs. The proteins levels of Fzd9 after osteogenic induction at 5, 10, 15, and 20 days in control and experimental groups. The expressions of Fzd9 protein in experimental groups were lower than those in control groups. C, E respectively means in control groups and experimental groups.

   Figure 4. Three-dimensional reconstruction of trabecular bone in the ROI. Trabecular bone in the ROI was reconstructed at day 20. Cortical bone was removed and green area means the reconstructed trabecular bone. There were more regular and denser trabecular bone in normal groups with than those in hyperlipidemic groups at day 20 (Fig. 4a). Histomorphometric quantification of trabecular bone in the ROI was shown in Fig. 4b-f. Scale bar, 1 mm. *P < 0.05.
Histomorphological quantitative analysis showed that the trabecular bone volume, the number of osteoblasts and the rate of bone formation in the hyperlipidemia groups decreased compared with the normal groups. In normal groups, BV/TV, Tb. Th and Tb. N were higher (BV/TV 1.37-folds, Tb. Th 1.22-folds, Tb. N 1.25-folds) than those in the hyperlipidemic groups, but Tb. S and Tbf were lower (Tb. S 0.62-folds, Tbf 0.64-folds) (P < 0.05) (Fig. 4b-f).

**Analysis of hard tissue slices**

The slices were stained with hematoxylin-eosin (HE) after implantation at day 20 (Fig. 5a). In the hyperlipidemic groups, the trabecular bone was sparser and more disordered than that in normal groups. The osteoblasts in hyperlipidemic groups were dramatically decreased 1.85-folds (Fig. 5b), and the osteoclasts increased 2.25-folds compared to the normal groups (Fig. 5c). Consistently, the implant showed decreased combination with bone in the hyperlipidemic groups and its BIC was lower than that in the normal groups (Fig. 5d).

**Expression of Fzd9 and Runx2 mRNA in bone tissue**

To further verify the impact of Fzd9 on hyperlipidemic rats, we ex-
amined the expression of Fzd9 and Runx2 in the bone around the implant. The results showed that Fzd9 and Runx2 in hyperlipidemic groups at days 5, 10, 15, and 20 were lower than that of normal groups (P<0.05) (Fig. 6).

**Western blot of Fzd9 in bone tissue**

To further confirm the expression of Fzd9 in bone tissue, the translation level of Fzd9 was examined by Western blot. Similarly, Western blot showed the reduction of Fzd9 protein in hyperlipidemic groups at days 5, 10, 15, and 20 in comparison to the normal groups (Fig. 7).

**Discussion**

In this study, we investigated that osteogenic differentiation of BMSCs in vitro and implant osseointegration in vivo were inhibited in hyperlipidemic conditions. During this process, the degree of Fzd9 expression was lower in hyperlipidemic conditions compared to the control. We speculated that hyperlipidemia could suppress implant osseointegration through Fzd9.

Hyperlipidemia plays a disruptive role in bone regeneration and repair, and it weakens the bone mechanical strength, as demonstrated by researchers. Higher serum lipid oxidized products caused by a HF diet are apt to increase PPARγ, which stimulates the process of adipogenesis and osteoclastogenesis and inhibits osteogenesis. Another study suggested that hyperlipidemia could also promote bone loss by decreasing parathyroid hormone (PTH) and Runx2, which undermines osteogenic differentiation. Herein, hyperlipidemia impairs the quality, quantity and healing potential of bone, which plays an important part in dental implant osseointegration. In our present study, we also confirmed that hyperlipidemia could inhibit implant osseointegration with fewer osteoblasts and trabecular bone.

Moreover, the hyperlipidemic rats showed decreased new trabecular bone with lower BV/TV, Tb. Th and Tb. N, but higher Tb. S and Tbf compared to the normal rats. These results indicated that hyperlipidemia destroyed the bone-to-implant interface and reduced implant osseointegration. The peri-implant bone formation and mineralization by osteoblasts resemble the processes of bone fracture healing. During fracture repair, osteoblasts form and accumulate collagenous matrix. Hyperlipidemia interrupts collagen processing as well as orientation and causes loss of local alignment, which might attenuate mechanical integrity and quality of bone. In addition to the mechanical quality of bone tissue around the interface, the area of the implant surface in intimate contact with mineralized bone tissue determines the quality of the bone-implant interface. And in our study, the hyperlipidemic rats showed decreased BIC than that in normal rats. Therefore, hyperlipidemia has a high risk of implant failure.

Fzd9, a specific Wnt receptor of the Frizzled family, participates in the Wnt signaling pathways and positively regulates new bone formation in fracture healing. Therefore, the lower expression of Fzd9 suppresses the initiation of Wnt signaling pathways, and further affects the transcription of Wnt downstream genes, such as Runx2. In this study, the expression of Fzd9 was suppressed at day 5, 10 and 15 and Runx2 was decreased at day 5 and 15 in vitro. Notably, the no significant difference in the expression of Fzd9 at day 20 in BMSCs might because Fzd9 was a main regulatory factor during the early stages of osteogenic differentiation. It is well known that genes generally can be formed into networks due to their interactions and one gene might be modulated by many regulatory factors, which might be the reason that there was no significant difference in the expression of Runx2 at day 10 and 20 in BMSCs. Consistently, ALP, ARS and ORO staining demonstrated the comprised osteogenesis in BMSCs. Runx2 in vivo was also downregulated by the HF diet, suggesting that the HF diet reduced the differentiation of osteoblasts which is consistent with the quantification analysis of HE staining in Fig. 5. Accordingly, the quality of the bone-to-implant interface was adversely affected and the number of osteoclasts increased in the 1mm bone around the implant. Therefore, we first demonstrated the negative impacts of lower Fzd9 in hyperlipidemic mice on implant osseointegration. As in previous studies, Fzd9 regulates bone information via noncanonical pathways, but another study showed that Fzd9 could activate β-catenin-dependent gene expression in 293T cells. The accumulation of β-catenin into the nucleus could mediate the transcription of Runx2. And in our study, the expression of Runx2 was decreased in hyperlipidemic conditions. Therefore, we confirmed that the lower expression of Fzd9 impairs osseointegration in hyperlipidemic conditions via the Wnt signaling pathway to downregulate Runx2.

Interestingly, because of the complexity of molecular regulatory mechanisms in the animal models, there might be many molecular regulatory mechanisms in the relation between osseointegration and hyperlipidemia. As our previous study showed, miR-29a-3p promoted the implant osseointegration of hyperlipidemic rats by targeting Wnt/β-catenin pathway-related Dvl2 and Fzd4. Additionally, Isg15, a ubiquitin-like modifier, was postulated to be one of the relevant downstream targets of Fzd9 in the regulation of bone formation, which provides important insights into other targets of Fzd9. Therefore, further studies should be conducted to explore some specific genes in canonical/β-catenin and noncanonical pathways to illustrate the relationship between Fzd9 and the two pathways in hyperlipidemia.

In conclusion, hyperlipidemia has an adverse impact on dental implant osseointegration, which might be regulated by Fzd9. And the lower expression of Fzd9 impairs osseointegration in hyperlipidemic conditions via the Wnt signaling pathway to downregulate Runx2. Further study will concentrate on the mechanism of Fzd9 in the Wnt signaling pathway and its upstream and downstream targets, which might provide more means to increase the success rate of implantation in patients with hyperlipidemia.

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**Conflict of Interest**

The authors declare that they have no conflicts of interest.

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