Heliyon 10 (2024) e25211

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Experimental analysis of femoral head intraosseous vascular anastomosis in the treatment of porcine subcapital femoral neck fractures

Wei Deng [a,1], Jiaming Wan [b,1], Dingsong Wang [a,1], Kailong Geng [a], Guangliang Zhang [c], Ruixing Hou [a,c,*]

[a] Suzhou Medical College of Soochow University, Suzhou, China
[b] Yangzhou University Medical College, Yangzhou, China
[c] Suzhou Ruihua Orthopedic Hospital, Suzhou, China

ARTICLE INFO

Keywords:
Femoral neck fractures
Femoral head necrosis
Animal experimentation
Blood vessels
Anastomosis

ABSTRACT

Introduction: Femoral neck fractures are challenging injuries associated with a compromised blood supply to the femoral head, leading to a high risk of avascular necrosis and poor clinical outcomes. This study aimed to investigate the efficacy of femoral head intraosseous vascular anastomosis in the treatment of porcine sub-capital femoral neck fractures.

Methods: Ten Landrace pigs were used as experimental animal models. The femoral head was completely removed after femoral neck sub-cephalic fracture. It was fixed on the medial side of the knee joint, and the blood supply to the femoral head was reconstructed by anastomosing the femoral head vessels. One week later, blood flow in the femoral head was observed by borehole, digital subtraction angiography examination, and hematoxylin and eosin staining. Further, terminal deoxynucleotidyl transferase-mediated dUTP nick-end labelling tests were performed to detect pathological changes in the femoral head.

Results: After one-week, digital subtraction angiography of the femoral head revealed a blood circulation rate of 70 %, and the blood seepage rate of the borehole was 80 %. Hematoxylin and eosin staining and terminal deoxynucleotidyl transferase-mediated dUTP nick-end labelling test results showed that necrosis of bone marrow cells in the experimental group was significantly improved compared to that in the control group.

Discussion: This study highlights the potential benefits of femoral head intraosseous vascular anastomosis in the treatment of porcine sub-capital femoral neck fractures. Further research and clinical trials are warranted to validate these findings and to explore the translational potential of this technique in human patients.

1. Introduction

Neck fractures are commonly observed in clinical practice. Due to aging population, the incidence of femoral neck fractures among elderly patients increases annually. Although the incidence of femoral neck fractures is lower in young adults [1], however, with

* Corresponding author. Suzhou Medical College of Soochow University, Suzhou, China.
E-mail address: hrx2023@126.com (R. Hou).
[1] WD and JW and DW contributed equally to this work.

https://doi.org/10.1016/j.heliyon.2024.e25211
Received 4 June 2023; Received in revised form 12 December 2023; Accepted 23 January 2024
Available online 30 January 2024

modern industry and economy, work-related accidents and injuries, road traffic accidents have made femoral neck fractures common in young adults. Femoral neck fractures in young adults are usually caused by high-energy injuries, a greater degree of displacement, and severe damage to the blood supply. The incidences of postoperative femoral head necrosis and non-union are relatively high. Femoral neck fractures in young adults have been described as unsolved fracture [2].

As young and middle-aged patients have greater requirement for hip function requiring higher service life of artificial joint prostheses, treatment of femoral neck fractures with internal fixation is preferred in young adults [3]. In young adults with femoral neck fractures, the necrosis rate of the femoral head after internal fixation is approximately 23 % [4]. Traditional treatments require a good reduction and firm fixation. However, they cannot effectively manage complications of avascular necrosis of the femoral head. Femoral head necrosis leads to femoral head collapse, joint deformity, pain, joint dysfunction, and poor quality of life [5]. To reduce the incidence of femoral head necrosis, various therapeutic techniques, such as bone transplantation, bone marrow stem cells, and platelet-rich plasma (PRP), with or without blood supply, have been combined [6–8], to achieve certain therapeutic effects. However, satisfactory treatments are not available.

The femoral head is mainly supplied by three groups of retinacular arteries [9]. The main cause of femoral head necrosis in displaced femoral neck fractures in young adults occurs when the displaced fractures damage the retinacular vessels and cut off the blood supply to the femoral head. These factors can lead to avascular necrosis of the femoral head [10]. Therefore, reconstruction of the femoral head vasculature may be a key factor in preventing femoral head necrosis. Zhao et al. reconstructed the blood supply to the femoral head by anastomosing the superior retinacular artery in a young adult with a femoral neck fracture [11]. After a 4-month follow-up period, the fracture healed completely without signs of femoral head necrosis. In subsequent studies [12], we examined retinacular artery injury after femoral neck fracture using digital subtraction angiography (DSA). In Garden III fractures, partial displacement of the complete fracture results in injury to the superior retinacular artery, whereas most of the inferior and anterior retinacular arteries remain intact. In Garden IV fractures, complete displacement resulted in injury to all retinacular arteries in all three groups. Wang et al. investigated the superior retinacular arteries in 10 cases of Garden III femoral neck fractures and found entrapment of the superior retinacular artery in six cases [13]. In such cases, arterial blood flow can be restored by reducing fractures. Garden IV fractures lead to complete injury of the femoral head blood supply, especially sub-capital femoral neck fractures, with a higher incidence of postoperative femoral head necrosis [14].

Based on the findings of the above report, Landrace pigs were used as experimental subjects. In the case of a completely displaced sub-cephalic femoral neck fracture, the blood supply to the femoral head was reconstructed by anastomosis of the blood vessels at the trophoblast hole of the femoral head, and patency was maintained for one week. Blood supply to the femoral head can be restored in the early stages by anastomosis of the blood vessels at the trophoblast hole of the femoral head. This animal model provides a new strategy for the treatment of displaced femoral neck fractures in young and middle-aged patients to facilitate subsequent clinical research.

2. Materials and methods

2.1. Materials

The experiments were conducted in accordance with the guidelines of the Animal Research Institute Committee of the Institutional Animal Care and Use Committee of Suzhou Ruihua Orthopedic Hospital. Animal care and procedures were approved by the Institutional Animal Care and Use Committee of the Suzhou Ruihua Orthopedic Hospital (approval number: RHGK2023043). All methods were performed in accordance with the ARRIVE guidelines.

Ten Landrace pigs were purchased from Wujiang Tianyu Biotechnology Co. Ltd. They weighed 100–110 kg, were aged 8–10 months, and were all male (Table 1). All pigs used in the experiment underwent animal quarantine and received quality certificates. A surgical microscope (Zhenjiang Zhuochuang Medical Technology Co., LTD.), microinstruments and microsutures (Ningbo Chenghe Microinstruments Factory), hollow nails (Tianjin Zhengtian Medical Device Co., Ltd.), Kirschner wires (Jiangsu Baiyi Medical Device Co., LTD.), an external fixator (Suzhou Easton Medical Device Co., Ltd.), and a DSA machine (Philips Medical Systems Nederland B. V. UNIQ FD20) were used.

Table 1
General characteristics of the animal model (n = 10) .

Number	Gender	Months	Weight (Kg)
PIG1	Male	9	100
PIG2	Male	8	100
PIG3	Male	9	110
PIG4	Male	10	101.8
PIG5	Male	10	110
PIG6	Male	9.5	104.4
PIG7	Male	9.5	104.5
PIG8	Male	9.5	100
PIG9	Male	10	110
PIG10	Male	10	108

3. Methods

The right hind limb was used as the experimental side and the left hind limb of the same pig was used as the control. In the previous pre-experiment, the right limb was used as the experimental side for sub-cephalic osteotomy of the femoral neck. Thereafter, the severed femoral head was fixed in situ and the trophoblast vessels at the junction of the femoral head and neck were anastomosed end-to-end. Due to the deep anatomical position, difficult surgical procedure, poor drainage, hematoma compression, poor postoperative

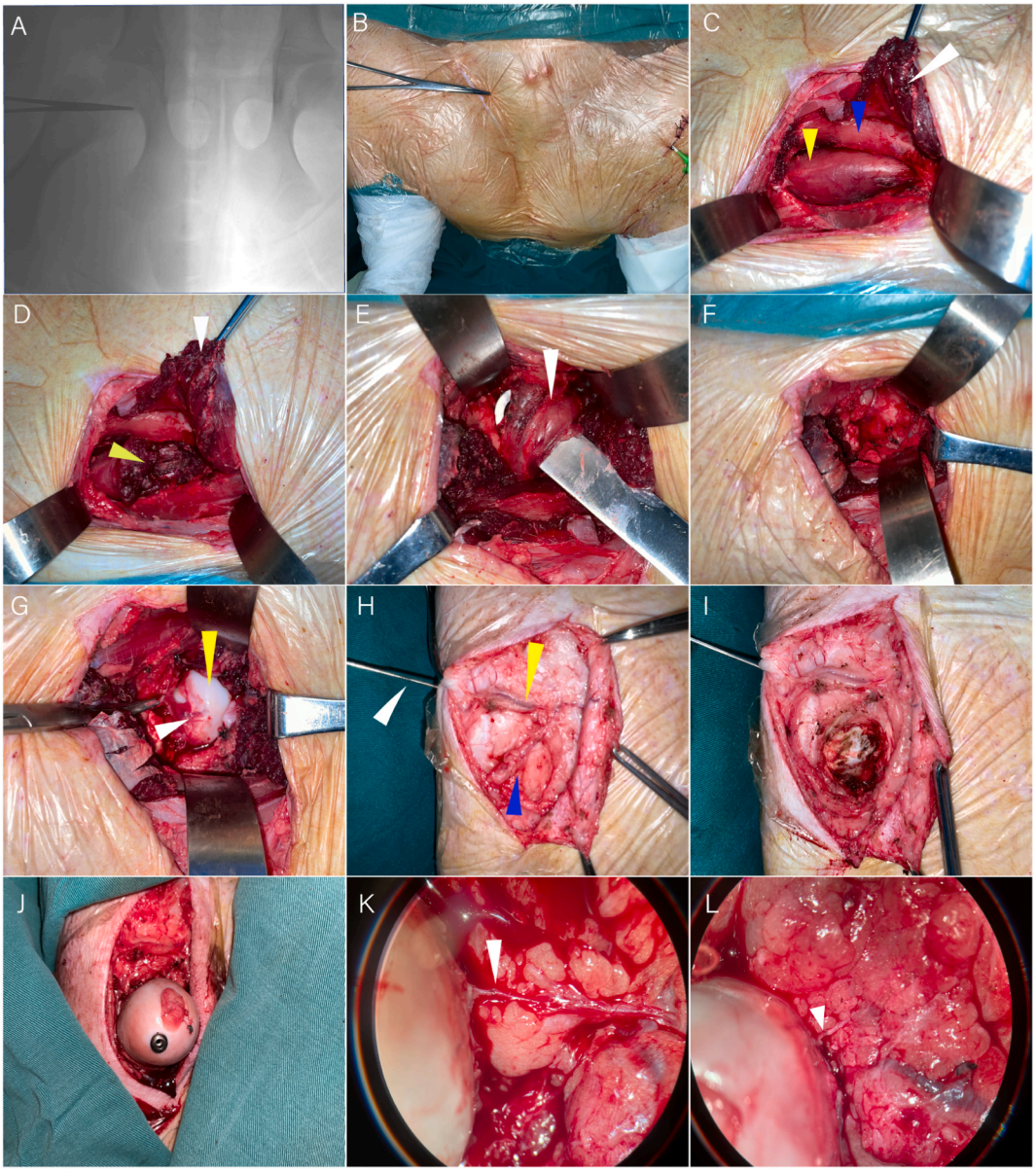

Fig. 1. A, **B.** Location of the shadow position of the femoral head on the body surface on radiography; **C.** The gracilis, sartorius, and pectineus muscles are indicated by the white, blue, and yellow arrows, respectively; **D.** The white arrow indicates the raised gracilis muscle and the yellow arrow indicates the pectineus muscle partially severed along the femoral lateral attachment, **E.** The iliopsoas muscle was cut and retracted. The iliopsoas muscle is indicated by the white arrow. **F.** Exposing the deep hip capsule after cutting the iliopsoas muscle; **G.** The deep femoral head was exposed after opening the joint capsule. The yellow arrow indicates the femoral head, the white arrow indicates the inferior retinacular artery at the femoral head-neck junction, **H.** White arrow indicates the level of knee joint space located by the Kirschner wire, yellow arrow indicates the branch of the saphenous artery, and blue arrow indicates the branch of the saphenous artery with vein, **I.** In the proximal inner side of the tibia, the soft tissue of the corresponding area was removed according to the sectional size of the femoral head until the bone surface; **J.** Fixation of the femoral head in the prepared recipient area with Kirschner wire and hollow nail; **K.** Anastomosed femoral head inferior retinacular artery; the white arrow indicates the vascular anastomosis; **L.** The anastomosed femoral head anterior retinacular artery and white arrow indicate the vascular anastomosis.

braking effect, and other factors, the experiment was unsuccessful. Therefore, the experiments were further improved. To overcome these limitations, the femoral head was removed from the experimental side and fixed to the medial side of the right proximal tibia. The saphenous arterial branch and accompanying vein were anastomosed end-to-end with the femoral head vessels to establish the femoral head arterial blood supply and venous reflux. After establishing the femoral neck-and-head fracture model on the control side, it was fixed on the medial side of the proximal left tibia without vascular anastomosis. The bilateral limb surgery was performed twice. In the first experiment, the right limb was tested, and the femoral head was removed one week later. In the second experiment, the left limb was tested, and the control femoral head was removed one week later.

3.1. Animal care and surgical preparation

Environmental requirements included indoor temperature of 24–26 °C and relative humidity of 50–60 %. The animals were fed with experimental pig feed (Jiangsu Medicience Co., LTD.) for one week after they had been transported to the laboratory and individually housed in iron cages, each measuring approximately 0.8 m × 2 m by 1.5 m in size. Prior to surgery, pigs were starved for 24 h and deprived of water for 12 h.

3.2. Anaesthesia care

Lumianning (2 mg/kg) (serazine hydrochloride injection 2 ml:0.2 g, Jilin Huamu Animal Health Products Co. Ltd.) was injected into the gluteal muscles. After 15–20 min, propofol (9–15 mg/kg) was administered via the marginal ear vein, and 10 ml of propofol was injected intravenously for induction. The pigs were fixed on a DSA operating table in the supine position. Intubation was performed, and the ventilator was connected after correct positioning. During the operation, 1.5–2 % sevoflurane (100 ml; Lunanbeite Pharmaceutical Co., Ltd.) was continuously inhaled, and intravenous anaesthesia was maintained with propofol and rocuronium (5 ml:50 mg; Zhejiang Xianju Pharmaceutical Co., Ltd.). The animals were prepared for aseptic surgery.

3.3. Surgical approach

Radiography was performed to locate the projection position of the body surface of the right femoral head (Fig. 1 A、B). centred on the surface projection of the femoral head, a surgical incision of about 15 cm was made anteromedially in the inguinal region, centred on the surface projection of the femoral head. Subsequently, the skin and subcutaneous tissues were cut open and separated from the muscle surfaces. At the origin of the pubis of the gracilis muscle, the width (about 3 cm) of the gracilis muscle was resected at the origin of the pubis. Further separation into the pectineus, sartorius, and adductor spaces was performed (Fig. 1C). The pectineus muscle was detached from the femoral attachment and lifted upwards to expose the iliopsoas muscle (Fig. 1 D, E). The iliopsoas muscle was severed from the femoral lesser trochanter attachment to expose the underlying hip capsule (Fig. 1F). The hip capsule was dissected (T-shaped) along the acetabular margin, exposing the femoral head (Fig. 1G), cutting the round ligament, and dislocating the femoral head. The femoral neck was cut at the junction of the femoral head and neck using a bone knife. The femoral head was then removed. Bone wax was used to seal the femoral neck osteotomy section and achieve haemostasis. The pelvic space was then filled with sterile gauze. After complete haemostasis, the gauze was removed and the incision was closed.

The nourishing holes of the posterior, inferior, and anterior retinacular arteries were identified based on the distribution position of the insertion head artery in the femoral head and neck [15], the nourishing holes of the posterior and inferior retinacular arteries and anterior retinacular artery were identified. Triangular fenestrations were performed, and the retinacular arteries were dissociated to an appropriate length for preservation.

A 12-cm arc-shaped incision was made at the centre, at the level of the right knee joint space, with the most obvious pulsation of the saphenous artery. The saphenous artery and accompanying vein were separated and exposed to identify the arteriovenous branch towards the medial side of the proximal tibia (Fig. 1H) [16]. After the vascular branch was freed, approximately 10 cm in length, the distal end was ligated, and the proximal end was lifted inward and wrapped with wet gauze. A mark was made on the proximal medial side of the tibia according to the size of the osteotomy surface of the femoral head. The soft tissues and periosteum were removed and the bone cortex was exposed (Fig. 1I). After complete haemostasis on the bone surface, the femoral head was fixed with a 1.5-mm diameter Kirschner wire and a 4.5-mm diameter hollow lag screw (Fig. 1J). The saphenous artery branch and the inferior retinacular artery were anastomosed end-to-end under a microscope (Fig. 1K).

After the arterial blood supply to the femoral head was restored, anastomosis was performed between the anterior retinacular artery and the saphenous artery with its venous tributaries (Fig.1L), and blood flow at the anastomosis was observed. Four stainless-steel pins (6 mm × 18 mm) were screwed into the proximal and distal ends of both tibiae. An external fixator system was installed to secure both hind legs, and the pig was placed in the lateral decubitus position (right side on top), with both external fixators attached to the cage to reduce movement. The second operation was performed one week after the first operation, and a control-side experiment was performed. The surgical approach, osteotomy, and fixation methods were the same as those used on the experimental side except for the vascular anastomosis.

3.4. Postoperative management

When the pigs were fully awake, they were returned to the animal house for feeding. The wound was disinfected with iodophor daily and wound drainage was observed. The wound drainage tube was removed two days later. Seven days after surgery, an

intravenous infusion pump was used every 24 h to drip the hibernation mixture (chlorpromazine hydrochloride 50 mg, Shanghai Wellhope Pharmaceutical Co., Ltd.; promethazine hydrochloride 50 mg, Shanghai Wellhope Pharmaceutical Co., Ltd.; petidine hydrochloride 100 mg, Yichang Renfu Pharmaceutical Co., Ltd.; 0.9 % sodium chloride injection 500 ml, Beijing Huaxia Shengsheng Pharmaceutical Company). Intramuscular injection of ceftiofuroxime sodium (0.5 g) (Chongqing Haimin Animal Pharmaceutical Co., Ltd) 1 g/d was performed to prevent wound infection. An intramuscular injection of papaverine hydrochloride (30 mg, 1 ml; Shenyang First Pharmaceutical Co., Ltd., Northeast Pharmaceutical Group) at 30 mg/q8 h was administered to prevent vasospasm. Heparin was injected intravenously 3 days after surgery (2 ml:12500 U, Changzhou Qianhong Biopharma Co., Ltd.) at 6250 U/Q8 h to prevent thrombosis. The enteral nutrient solution was administered orally at 1 L/q12 h daily (Nengquan, Wuxi Nutricia Pharmaceutical Co., Ltd.) to supplement nutrition.

4. Observation index

4.1. General observation

The diameter (Fig. 2A), height (Fig. 2B), and mass of the femoral head were measured. The outer diameter of the retinacular artery at the trophoblast hole was measured at a microscale using a microscope (Fig. 2C and D). After vascular anastomosis was completed, the vascular clamp was removed to observe distal vascular filling and arterial pulsation. The femoral head was drilled with 2.0 mm Kirschner wire at a depth of 1.0–1.5 cm. Normal heparin saline was used to wash the drilled hole and bleeding from the drilled hole was observed (Fig. 2E). Anastomotic patency was observed one week postoperatively.

4.2. Radiographic observation

DSA was performed one week postoperatively (Fig. 2F). A reverse saphenous artery puncture was performed in the lower part of the leg, distal to the vascular anastomosis. An indwelling needle no. 22G was inserted to connect the angiography tube. Angiography was performed by injecting an iodoferol contrast agent (100 ml:32 g; Tyco Healthcare) into the tube with a high-pressure injection pump (150 PSI; velocity of 3 ml/s; total volume of 8 ml). Circulation of the contrast agent in the femoral head was observed to understand the blood supply to the femoral head. After conducting the relevant experiments, euthanasia was performed via intravenous injection of Zoletil mixture, followed by exsanguination.

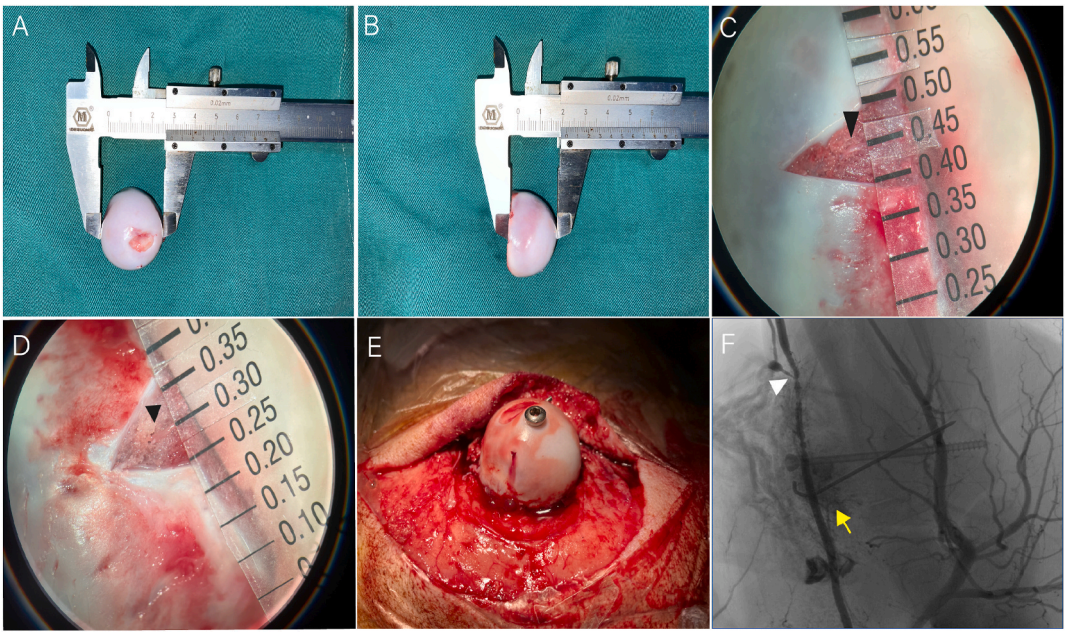

Fig. 2. A. The diameter of the experimental femoral head was 29 mm; **B.** The thickness of the femoral head on the experimental side was 21.7 mm; **C.** The diameter of the femoral head inferior retinacular artery on the experimental side was 0.45 mm; **D.** The diameter of the femoral head anterior retinacular artery on the experimental side was 0.30 mm; **E.** Blood oozing at the borehole after the completion of the retinacular arterial anastomosis of femoral head; **F.** DSA angiography was performed one week after surgery, and contrast agent was circulating in the femoral head. The white solid arrow represents the saphenous artery, the yellow dotted line represents the femoral head, and the white dotted line arrow represents the anastomosis.

4.3. Histological observation

The femoral head specimens were fixed in a 10 % formaldehyde solution for at least 48 h. The femoral head was evenly divided into four pieces along the central axis. The severed femoral head tissues were decalcified with 10 % EDTA solution for 4 weeks. Femoral head specimens were cut into 4-mm thick tissues, fully dehydrated, embedded with paraffin, and made into 4-μm paraffin sections. Paraffin sections were stained with hematoxylin (and) to observe the morphology of bone trabecular cells, bone cells, chondrocytes, and bone marrow cells. Paraffin sections of the femoral head were prepared using the TUNEL assay. After the paraffin sections of the femoral head were fully dehydrated, protease K was added onto the section tissue (ST532 Biyotime Biotechnology Co., LTD.). After protease K was washed, the TUNEL assay solution (C1089 Biyotime Biotechnology Co., LTD.) was added for labelling, and the apoptosis of cells in the femoral head was observed under fluorescence microscope.

4.4. Data analysis

The diameter, length, and mass of bilateral femoral heads were expressed by mean ± standard deviation. Student's t-test was used to assess differences in the quality and size of the femoral head between the experimental and control groups. The results of borehole oozing and DSA examination one week after femoral head vascular anastomosis were defined as a binary response (yes/no). Fisher's exact test was used to assess the association between bilateral femoral head drilling and DSA. The number of lipid cells present in pores of bone trabeculae after HE staining of bilateral femoral head and the percentage of necrotic areas of bone marrow cells detected by TUNEL were expressed as mean ± standard deviation. The Student's t-test was used to determine the difference in the number of femoral head adipocytes and the percentage of necrotic bone marrow cells between the experimental and control groups. All data were analysed using SPSS software (version 17.0; Chicago, IL, USA), and p-values <0.05 were considered statistically significant.

5. Results

The survival rate of the ten pigs was 100 % after the experiment. No complications such as wound infection occurred in any of the experimental pigs, and relatively complete experimental data were obtained.

5.1. The outcomes of general observation

The mass of the experimental femoral head was 26.26 ± 3.10 g, and that of the control femoral head was 26.02 ± 3.16 g ($P = 0.414$). The diameter of the experimental femoral head was 32.18 ± 2.80 mm, and that of the control femoral head was 32.10 ± 2.83 mm ($P = 0.514$). The thickness of the femoral head on the experimental side was 23.64 ± 2.58 mm, and that on the control side was 23.56 ± 2.77 mm ($P = 0.700$). The differences between the above data were not statistically significant. The diameter of the inferior retinacular artery at the trophoblast hole of the femoral head was 0.47 ± 0.05 mm, and that of the anterior retinacular artery at the trophoblast hole of the femoral head was 0.35 ± 0.06 mm (Table 2). The anterior retinacular artery was shorter than the inferior retinacular artery. These findings were consistent with the results of our previous study, and the vessel diameter (>0.3 mm) fully supported the need for vascular anastomosis.

Table 2
Characteristics of the femoral head.

Variable		PIG1	PIG2	PIG3	PIG4	PIG5	PIG6	PIG7	PIG8	PIG9	PIG10	$\bar{x}$±SD	P Value
mass (g)	experimental group	24.4	23.3	28.8	23.9	29.2	30.4	30.7	23.7	24.7	23.5	26.26 ± 3.10	0.414
	Control group	24.1	23.1	28.3	23.6	28.6	30.1	31.2	24	24.2	23	26.02 ± 3.16	
diameter[a] (mm)	experimental group	31	28.3	34.3	30.5	34.4	35.7	36	30	32.6	29.0	32.18 ± 2.80	0.514
	Control group	30.2	28	33.6	31.3	34.5	35.6	36.5	30.5	31.6	29.2	32.10 ± 2.83	
thickness† (mm)	experimental group	21.8	20.8	25.7	21.6	25.6	26.7	27.8	21.4	23.5	21.7	23.64 ± 2.58	0.7
	Control group	21.6	20.5	26.1	22	25.3	26.8	28.3	21.7	21.9	21.4	23.56 ± 2.77	
Inferior retinacular artery diameter‡ (mm)	experimental group	0.5	0.4	0.5	0.4	0.5	0.55	0.45	0.4	0.5	0.45	0.47 ± 0.05	–
Anterior retinacular artery diameter‡ (mm)	experimental group	0.35	0.3	0.4	0.4	0.3	0.45	0.3	0.3	0.35	0.3	0.35 ± 0.06	–

[a]Maximum diameter of the femoral head osteotomy surface. †Represents the distance from the highest point of the femoral head along the central axis of the femoral neck to the osteotomy plane. ‡Represents the outer diameter of the retinacular artery at the nutrient hole of the femoral head.

6. The outcomes of hemoperfusion of femoral head

One week after the surgery, the femoral head on the experimental side was tested for blood circulation. During DSA, we found that three patients had no contrast agent in the femoral head. During the operation, we drilled the femoral head again and found no bleeding in two cases. In one case, no contrast agent was present on DSA examination of the femoral head. However, bleeding occurred during the drilling. The vascular anastomosis was observed in the triangular window under a microscope; the vascular anastomosis remained unobstructed. The possible reasons for this were the sudden increase in intravascular pressure during DSA or the stimulation of blood vessels by the contrast agent itself. This irritation caused vasospasms, which resulted in false-negative DSA results. Two other cases of femoral head without blood flow on the experimental side were observed under a microscope. Embolisms were present in the anastomoses of the inferior and anterior retinacular arteries. These vessels contained long thrombus segments in the lumen. This may be due to poor vascular bed conditions, vascular tortuosity caused by low vascular tension, or local hematoma compression (Table 3).

6.1. Histopathology

Light microscopy revealed that the morphology of bone cells in the trabecular bone of the femoral head was similar between the anastomosed and non-anastomosed groups. Furthermore, the number of lacunar cells did not differ significantly (Fig. 3A, ~C, G, ~I). Upon observation of the bone marrow between the bone trabeculae, more fat cells were present on the anastomosed side, with no obvious accumulation of bone marrow cells (Fig. 3B). Fewer fat cells and a large amount of abnormal accumulation of bone marrow cells were observed in the non-anastomosed femoral head (Fig. 3H). Four sections of each femoral head were observed under a 200x optical microscope. Five fields were randomly selected for observation and the number of adipocytes between the bone trabeculae was counted. The number of adipocytes between trabecular bones of anastomosed femoral head was 66.88 ± 6.03 adipocytes, and that among trabecular bones of the non-anastomosed femoral head was 25.13 ± 3.31 adipocytes ($P < 0.001$)(Fig. 3M). No significant differences in the morphology and number of chondrocytes were observed between the anastomosed and non-anastomosed femoral heads (Fig. 3D–F, J ~ L).

The TUNEL test was performed on the femoral head of the eight experimental sides and their corresponding control sides. Apoptotic bone marrow cells were labelled with red fluorescence. Five fields were randomly selected for each paraffin section, and the percentage of areas of red fluorescent-labelled bone marrow cells (necrotic bone marrow cells) in the bone marrow cavity in each field was calculated. In the anastomosed femoral heads, fewer dead bone marrow cells were present in the bone marrow lumen than in the control femoral heads (Fig. 4A and B). The percentage of areas with dead bone marrow cells was 19.27 ± 11.10 %. In the non-anastomosed femoral heads, more dead bone marrow cells were present in the lumen of the femoral heads (Fig. 4C and D). The percentage of areas with dead bone marrow cells was 47.21 ± 10.11 %. The difference between the two groups was statistically significant ($P < 0.001$) (Fig. 4E)

7. Discussion

7.1. The significance of this study

Displaced femoral neck fractures in young adults are primarily caused by violent injuries. This type of fracture extensively damages the blood supply to the femoral head extensively [17]. Damage to the femoral head blood supply is especially severe in sub-capital femoral neck fractures. Femoral head necrosis tends to occur when fractures are treated with traditional internal fixation. To improve the blood supply to the femoral head after femoral neck fracture and reduce the incidence of necrosis, many researchers have attempted to reconstruct the blood supply to the femoral head using methods such as the femoral quadratus bone flap, vascularized iliac crest graft, and vascularized greater trochanter bone flap [6,8,18–20]. These surgical techniques offer some therapeutic benefits in the treatment of femoral neck fractures and the prevention of femoral head necrosis. However, they also increase the risk of injury to the donor site. Zhao et al. reconstructed the femoral head blood supply to treat femoral neck fractures in young adults by anastomosing the superior retinacular arteries and veins [11]. The patient was followed-up, and a satisfactory therapeutic effect was observed.

Table 3
Blood circulation of femoral head after one week.

Number	Drilling			DSA		
	Experimental group	Control group	P Value	Experimental group	Control group	P Value
PIG1	Y	N	0.001	Y	N	0.003
PIG2	Y	N		Y	N	
PIG3	N	N		N	N	
PIG4	Y	N		Y	N	
PIG5	Y	N		Y	N	
PIG6	Y	N		N	N	
PIG7	Y	N		Y	N	
PIG8	N	N		N	N	
PIG9	Y	N		Y	N	
PIG10	Y	N		Y	N	

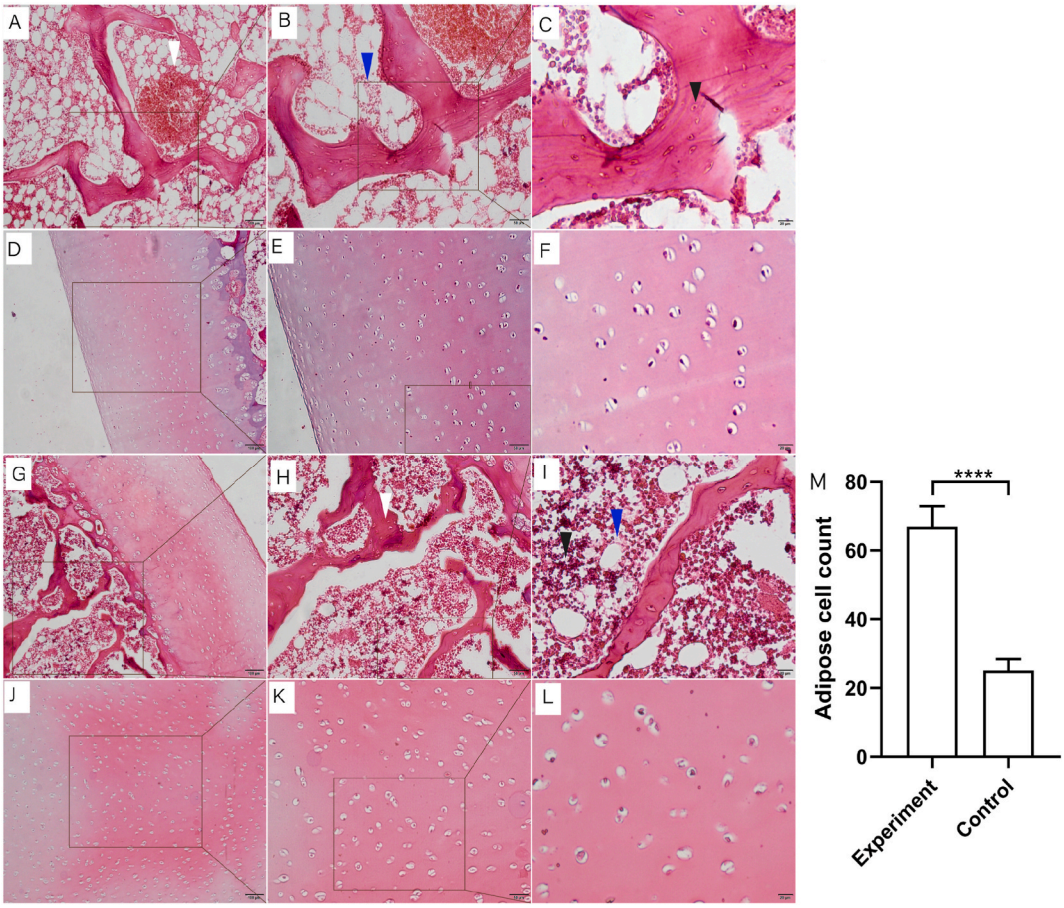

Fig. 3. A- C. Changes in bone trabeculae and bone marrow in the femoral head at the experimental side. The white arrow indicates that the vascular lumen is filled with red blood cells, the black arrow indicates bone cells, and the blue arrow indicates bone marrow cells; **D-F.** Changes of femoral head cartilage on the experimental side; **G-I.** Changes in trabecular bone and bone marrow in the control femoral head. The white arrow indicates trabecular bone, the blue arrow indicates adipose cell, and the black arrow indicates marrow cell; **J-L.** Changes of femoral head cartilage on the control side. **M.** The adipose cells in the trabecular space of the femoral head in the experimental group were significantly more than those in the control group ($P < 0.001$).

According to autopsy studies, the distance from the insertion point of the retinacular artery to the femoral head and neck junction is 2–4 mm [20–22]. This study provides evidence for the possibility of vascular anastomosis in patients with sub-capital fractures of the femoral neck.

In previous experimental studies [15], our research group found that the distance from the entry point of the retinacular artery of the femoral head and neck to the femoral head and neck junction and its distribution position in the femoral neck of pigs were similar to those in humans. All femoral neck fracture models used in this study were sub-capital femoral neck fractures. During the operation, the corresponding nourishing pores were identified according to the approximate position of the distribution of the inferior and anterior retinacular arteries to avoid blind searching. Triangle-shaped fenestrations were created at the nourishing aperture, and the epiphyseal artery in the femoral head was appropriately dissociated to facilitate vascular anastomosis. One week after the vascularized anastomosis of the femoral head was completed, we drilled the femoral head and observed a blood seepage rate of 80 %. DSA was performed on the femoral head vessels and the vascular patency rate was 70 %. Most femoral heads maintained unobstructed blood flow one week after the operation. Compared with internal fixation, this treatment can significantly improve blood supply to the femoral head in the early stages after a femoral neck fracture.

Early pathological changes in osteonecrosis occur primarily in the bone marrow [23]. No significant differences in the trabecular bone, osteocytes, or chondrocytes were observed between the experimental and control groups using H&E staining. Regarding the bone marrow tissue between the trabeculae, the femoral head on the control side was filled with several bone marrow cells. Relatively few bone marrow cells were present on the experimental side of the femoral head between the trabeculae. This could be attributed to the supply of nutrients to the femoral head on the control side via local oozing. In this group, the femoral heads had poor venous return, resulting in severe congestion of the bone trabeculae and an abnormal accumulation of bone marrow cells. The TUNEL assay confirmed that the number of apoptotic cells in the trabecular bone of the femoral head on the control side was significantly higher than that on the experimental side. Therefore, this treatment may reduce the incidence of femoral head necrosis to a certain extent, but further

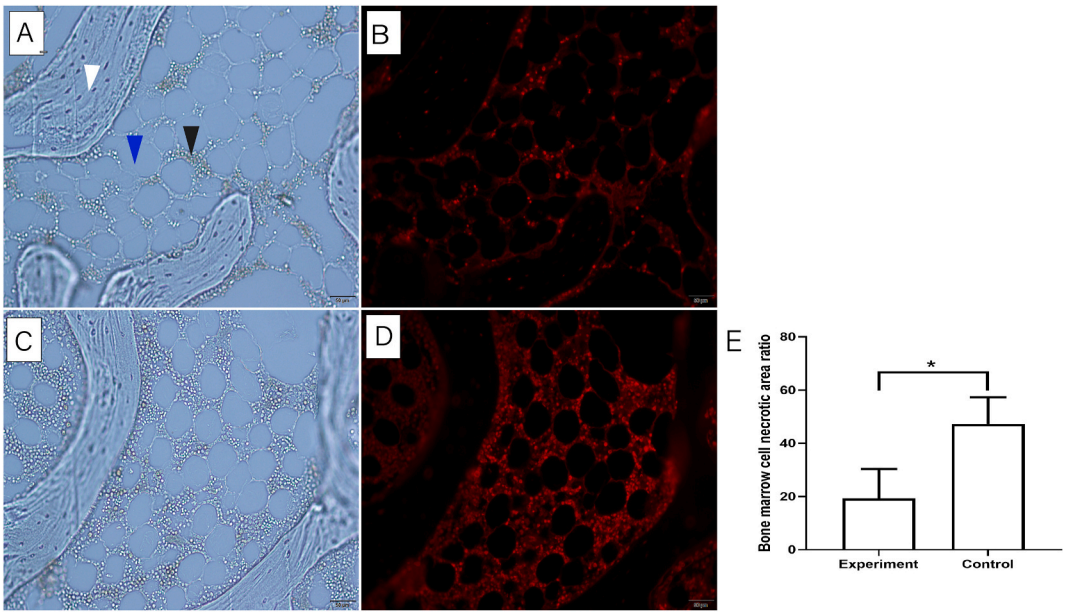

Fig. 4. A. Observation under bright field of experimental group femoral head. The white arrow represents bone trabecular bone, the blue arrow represents fat vacuoles, and the black arrow represents bone marrow cells**; B.** Observed under fluorescence microscope. Necrotic apoptotic bone marrow cells are shown in red; **C.** Bright field observation under the control group femoral head; **D.** Fluorescence microscope observation on the control side of the femoral head**; E.** The necrosis and apoptosis of bone marrow cells in the femoral head in the experimental side were more significantly improved than that in the control side ($P < 0.001$).

studies are needed to confirm this finding.

7.2. The advantage of the described approach

The main advantage of this study is that cortical fenestrations were created in the arterial trophoblast of the femoral head to anastomose the supporting vessels for the reconstruction of the arterial blood supply and venous reflux of the femoral head. This method can improve femoral head ischemia and prevent venous return stagnation after completion of arterial anastomosis. Anastomosis of the femoral head retinacular vessels is not only suitable for the treatment of sub-capital femoral neck fractures, but also for the treatment of cervical and basal femoral neck fractures. Due to the network distribution of blood vessels in the femoral head [24], a group of retinacular vessels with relatively large diameters was selected for anastomosis. The anastomosed femoral head retinacular vessels supply blood to the entire femoral head through the femoral head [25]. Concurrently, the anastomosis of the anterior retinacular artery and proximal return vein ensures the return of blood to the femoral head. For internal fixation, a 4.5-mm hollow lag screw was used to fix the femoral head. Concurrently, a 1.5-mm diameter Kirschner wire was used for anti-rotation. Owing to the existence of a network structure in the central region of the femoral head, more anastomoses of vascular traffic and a strong compensatory ability were present. Both implants were located in the central area of the support, with the vessels away from the anastomosis. These fixations prevented damage to the main epiphysiological vessels of the femoral head. Therefore, this fixation method causes minimal damage to the femoral head vessels.

7.3. The limitations of this study

First, risk period for vascular embolism is approximately one week after performing vascular anastomosis. However, we did not continuously monitor hemoperfusion of the femoral head dynamically, and were unable to intervene within one week of surgery. However, relatively mature technologies have been developed to monitor femoral head blood flow [26,27]. This type of defect can be completely avoided if femoral head vascular anastomosis is performed on the human body. Second, we draw conclusions from the failures of previous pre-experimental cases. In the early stages, we performed in situ replantation of sub-capital femoral neck fractures. Owing to the deep location of the operation, the operation time was long, and blood loss was extensive. Typically, the mortality rate of experimental pigs was. Therefore, ectopic replantation was considered, which effectively prevented the problems of deep anatomical positioning, difficult operation, long operation time, and extensive blood loss. Therefore, this method cannot simulate the actual process of sub-capital femoral neck fracture healing. Further, the study period was short and the technique focused on improving the blood supply to the femoral head in the early stages of femoral neck fractures. Regarding fracture healing, further research is needed to improve the mid-and late blood supply to the femoral head. Third, the sub-capital femoral neck fracture model established in this study was obtained by sharp osteotomy along the head-neck junction using a bone knife with a width of 30 mm. To some extent, the model

was different from a real sub-capital femoral neck fracture. In clinical practice, standard sub-cephalic femoral neck fractures are less common and are replaced by femoral head and neck fractures [28]. The fracture line in this type of fracture starts at the head-neck junction above the femoral neck and ends at the middle or base of the femoral neck below the femoral neck. In the case of a simple displaced fracture, the superior retinacular artery located on the upper side of the femoral neck is damaged only at the displaced fracture site and vascular anastomosis can be performed directly. In cases of compression or avulsion fractures, long-segment injury of the superior retinacular arteries may be present. Compared to the retinacular arteries of the trophoblast hole at the femoral head and neck junction, the epiphyseal vessels in the femoral head have relatively small diameters. Consequently, vascular anastomosis becomes more difficult. In addition, in-situ anastomosis using residual blood vessels is less likely to be successful. In such cases, other blood vessel branches must be transferred for anastomosis. In the lower part of the femoral neck, the fracture site is near the base of the femoral neck, and the rupture location of the inferior retinacular artery may be at the base of the femoral neck, rather than at the femoral head and neck junction. Compared to the vascular anastomosis method used in this study, inferior retinacular artery anastomosis is simpler.

8. Conclusion

In this study, blood supply to the femoral head after a sub-capital femoral neck fracture was reconstructed by anastomosing the retinacular vessels at the point where the retinacular vessels entered the femoral head. This method can guarantee blood supply to the femur in the early stage after internal fixation of femoral neck fractures and effectively improve blood return to the femoral head. To prevent femoral head necrosis effectively, further long-term follow-up studies with larger sample sizes should be conducted to verify these findings. Concurrently, related factors, such as the time node of femoral head-bearing weight and stability of fracture fixation, should be considered. This study proposes a new strategy for clinical treatment and needs to be confirmed by further clinical studies.

Funding

Suzhou Science and technology planning project (SYSD2019056).

Ethics declarations

The experiments were conducted in accordance with the guidelines of the Animal Research Institute Committee of the Institutional Animal Care and Use Committee of Suzhou Ruihua Orthopedic Hospital. Animal care and procedures were approved by the Institutional Animal Care and Use Committee of the Suzhou Ruihua Orthopedic Hospital (approval number: RHGK2023043). All methods were performed in accordance with the ARRIVE guidelines.

Data availability statement

All data and materials are included in the article.

CRediT authorship contribution statement

Wei Deng: Writing – original draft, Validation, Project administration, Investigation, Formal analysis, Conceptualization. **Jiaming Wan:** Supervision, Software, Project administration, Investigation, Formal analysis, Data curation, Conceptualization. **Dingsong Wang:** Writing – original draft, Resources, Investigation, Funding acquisition, Data curation, Conceptualization. **Kailong Geng:** Validation, Software, Resources, Investigation, Data curation. **Guangliang Zhang:** Visualization, Validation, Software, Funding acquisition, Formal analysis. **Ruixing Hou:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Not applicable.

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