Supplementary material

Table i. Summary of osteoporotic metaphyseal fracture animal models

No.	Study	Year	Animal	Method, time to establish osteoporosis, and technique to confirm osteoporosis	Site of metaphyseal fracture	Type of fracture	Fixation and % success of fracture	Radiology/histology evidence of healing	End points (days)	Strengths and weaknesses	Utility of model in fracture healing
1	Alt et al[38]	2016	Goat	OVX on mature 3-yr-old Chinese mountain goats with low-calcium diet, 6 mths for establishment; pQCT	Lumbar spine, bilateral iliac crest, distal femur	Drill hole, 8 mm on both iliac crests and distal femur; 5 mm on lumbar vertebra	None; 100% success rate (3/3, n = 3)	HR-pQCT: increased trabecular number and decreased trabecular spacing at margins of defect. Histology: defects filled with granulation tissue or lipid rich bone marrow. Surrounding bone lining cells become active osteoblasts producing collagen into direction of defect	42	Strengths: haversian system similar to that found in humans; easy to perform drill hole. Weaknesses: drill hole defects do not represent clinically relevant scenario; less efficacious in terms of cost and availability, housing and spatial requirements, manageability, and reproducible results; second line for FDA-approved osteoporotic models	Study of biomaterials, e.g. HA/col-1 and HA to fill into defects. HA/col-1 had the highest connectivity density and highest number of trabeculae compared to HA and an empty defect. HA had significantly higher new bone formation compared to the empty defect. Histology revealed good biocompatibility without inflammatory reaction for both implants
2	Komrakova et al[31]	2016	Rat	OVX on 3-mth-old SD-rat with soy-free diet, 8 wks for establishment; micro-CT	Bilateral proximal tibia	Complete osteotomy	Plate and screws; no details on success	CT: callus bridging of osteotomized bone ends from wk 3 and onwards. Healed at 5 wks	42	Strengths: clinically relevant model; OVX rats are FDA-approved model; easy to handle, reproducible. Weaknesses: bilateral osteotomy has negative influence in weight-bearing and welfare concerns	Study of potential therapeutic agents and noninvasive interventions, e.g. PTH and WBV. WBV, in addition to PTH, increased the cortical and callus width, as well as biomechanical properties. However, WBV with SR had no additional advantage compared with SR alone
3	Komrakova et al[30]	2015	Rat	OVX on 3-mth-old SD-rat with soy-free diet, 8 wks for establishment; micro-CT	Bilateral proximal tibia	Complete osteotomy	Plate and screws; no details on success	Histomorphometry: first osseous bridging at day 25	35	Strengths: clinically relevant model; OVX rats are FDA-approved model; easy to handle, reproducible. Weaknesses: bilateral osteotomy has negative influence in weight-bearing and welfare concerns	Study of potential therapeutic agents, e.g. SR. Results showed that oral SR as a therapeutic and prophylactic agent increased total BMD, callus area and density, accelerated osteotomy bridging, and callus formation at wks 2 and 3 of osteoporotic fracture healing. SR as a pure therapeutic agent enlarged the callus area and improved callus formation at wk 5 of fracture healing
4	Tao et al[23]	2015	Rat	OVX on 3-mth-old SD-rat, 3 mths for establishment; based on previous studies	Distal femur	Drill hole, 3 mm on distal femur	None; 95% (57/60, n = 60); 3 excluded due to anaesthetic accident, infection, and inaccurate fracture site, respectively	Micro-CT: new bone formation progressively from 4 to 8 wks at the margins of the defect. Histology: new woven bone limited and confined to the margins of the drill hole with no bone formation at the centre of the defect. New bone extend to the direction of the defect	28, 56	Strengths: OVX rats are FDA-approved model; easy to handle, reproducible; easy to perform drill hole. Weaknesses: drill hole defects do not represent a clinically relevant scenario	Study of potential therapeutic agents, e.g. PTH in combination with β-tricalcium phosphate. PTH in combination with β-tricalcium phosphate allowed the bone defect created to have significantly better bone healing

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5	Stuermer et al[28]	2014	Rat	OVX on 3-mth-old SD-rats, 8 wks for establishment; based on previous studies	Bilateral proximal tibia	Complete osteotomy	Plate and screws; no details on success	Micro-CT: healing at 5 wks, callus formation had decreased density compared to the sham group	35	Strengths: clinically relevant model; OVX rats are FDA-approved model; easy to handle, reproducible. Weaknesses: bilateral osteotomy has negative influence in weight bearing and welfare concerns	Study of potential therapeutic drugs and intervention, e.g. WBV with oestrogen or raloxifene. Combination treatment of WBV with oestrogen or raloxifene enhanced fracture trabecular density, with WBV and raloxifene also enhancing fracture stiffness and endosteal bone
6	Thormann et al[32]	2014	Rat	OVX on 4- to 5-mth-old SD-rat with multi-deficiency diet, 3 mths for establishment; DXA scan	Distal femur	3 mm complete triangular defect	Plate and screws; no details on success	Micro-CT and histology: bridging of cortices and consolidation at 6 wks	42	Strengths: clinically relevant model; OVX rats are FDA-approved model; easy to handle, reproducible. Weaknesses: technically difficult surgery	Study of potential biomaterials to place into defect
7	Ibrahim et al[22]	2014	Rat	OVX on 3-mth-old SD-rat, 2 months for establishment; micro-CT	Proximal tibia	Complete osteotomy	Plate and screws; no details on success	CT: small amount of mineralized callus over the fracture site at 4 wks	28	Study of potential therapeutic drugs, e.g. lovastatin and tocotrienol. Combined treatment of lovastatin and tocotrienol showed significantly higher	Study of potential therapeutic drugs, e.g. lovastatin and tocotrienol. Combined treatment of lovastatin and tocotrienol showed significantly higher callus volume and strength compared to control group. Both lovastatin and tocotrienol alone had significantly higher callus strength than control but not for callus volume
8	Stuermer et al[27]	2013	Rat	OVX on 3-mth-old SD-rats with phytoestrogen-free diet, 10 wks for establishment; based on previous studies	Bilateral proximal tibia	Complete osteotomy	Plate and screws; 97% (35/36, n = 36), 1 had intraoperative fibula fracture	CT: adequate fracture healing at 5 wks	35	Strengths: clinically relevant model; OVX rats are FDA-approved model; easy to handle, reproducible. Weaknesses: bilateral osteotomy has negative influence in weight-bearing and welfare concerns	Study of potential therapeutic agents, e.g. raloxifene and oestrogen. Raloxifene and oestrogen both had supporting effects in fracture healing in rats in late phase of osteoporosis by improving callus structure and mechanical properties
9	Bindl et al[37]	2013	Sheep	Hypothalamic-pituitary disconnection on adult Merino sheep, 6 mths for establishment; pQCT	Distal femur	Partial osteotomy	None; 100% (8/8, n = 8)	CT: decrease in newly formed bone in osteoporotic sheep compared with control group by 30% in BMD and 36% in bone volume/total volume Histology: decrease new bone content and remaining cartilage in the osteoporotic group	56	Strengths: haversian system similar to that of humans; easy to perform partial osteotomy. Weaknesses: partial osteotomies do not represent clinically relevant scenario	Study of potential therapeutic agents

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10	Thormann et al[24]	2013	Rat	OVX on 14-wk-old SD-rats and multi-deficiency diet; 3 mths for establishment; DXA	Distal femur	4 mm complete triangular defect	Plate and screws; 87% (39/45, n = 45), 3 died from anaesthesia, 1 from OVX, 2 from femur surgery	Histology revealed almost a lack of osteoid and little cartilage formation, with predominantly fibrous tissue	42	Strengths: clinically relevant model; critical size defect; OVX rats are FDA-approved model; easy to handle, reproducible. Weaknesses: technically difficult surgery	Study of potential biomaterials that are placed into defect, e.g. SrCPC and CPC. There was significantly higher bone formation in bone with SrCPC compared to CPC and the empty defect. There was higher bone formation at the biomaterial-tissue interface with SrCPC
11	Alt et al[7]	2013	Rat	OVX; DXA	Distal femur	3 mm and 5 mm complete triangular bone defect	Plate and screws; 93%, 3 mm (12/13, n = 13), 5 mm (14/15, n = 15), 1 died after OVX, and 1 died from anaesthesia	Micro-CT and histology: complete mineralized bridging of the 3 mm wedge defect. 5 mm defect filled with fibrous tissue and did not achieve healing	42	Strengths: clinically relevant model; critical size defect; OVX rats are FDA-approved model; easy to handle, reproducible. Weaknesses: technically difficult surgery	Study of potential biomaterials
12	Komrakova et al[34]	2013	Rat	OVX on 3-mth-old SD-rat, 8 wks for establishment; micro-CT	Bilateral proximal tibia	Complete osteotomy	Plates and screws; no details on success	Histology: osseous bridging at 26 days	35	Strengths: clinically relevant model; OVX rats are FDA-approved model; easy to handle, reproducible. Weaknesses: bilateral osteotomy has negative influence in weight-bearing and welfare concerns	Study of potential therapeutic interventions e.g. WBV. Between 35Hz and 50Hz vertical WBV, there was an acceleration in osteotomy bridging, increase in muscle weight, improvement in cortical and callus densities, and an enlargement of callus area and width. Horizontal WBV showed no positive or negative effects
13	McDonald et al[35]	2012	Rat	OVX on SD-rat with age unspecified; 4 wks to simulate early osteoporosis; DXA	Bilateral proximal tibia	Drill hole, 3 mm on bilateral proximal tibia	None; no details on success	CT and histology: progressive healing to 3 wks	7, 14, 21	Strengths: OVX rats are FDA-approved model; easy to handle, reproducible; drill hole defects are easy to perform. Weaknesses: bilateral osteotomy has negative influence in weight-bearing and welfare concerns; drill hole defects do not represent a clinically relevant scenario	Study of potential therapeutic agents, e.g. Scl-Ab. There was significant increase in bone formation through fluorochrome labelling with Scl-Ab treatment. Scl-Ab showed an increase in bone volume in the defect in both histology and micro-CT
14	Kolios et al[29]	2010	Rat	OVX on 3-mth-old SD-rats with phytoestrogen-free diet, 10 wks for establishment; based on previous studies	Bilateral proximal tibia	Complete osteotomy	Plate and screws; 89% (32/36, n = 36), 4 rats had intra-operative fibula fracture	CT: achieved adequate fracture healing at 5 wks	35	Strengths: clinically relevant model; OVX rats are FDA-approved model; easy to handle, reproducible. Weaknesses: bilateral osteotomy has negative influence in weight-bearing and welfare concerns	Study of potential therapeutic agents e.g. oestrogen or alendronate as prophylactic therapy. Administration of oestrogen improved the biomechanical properties of the callus but alendronate did not help in bone healing

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15	Kolios et al[26]	2010	Rat	OVX on 3-mth-old SD-rats with phytoestrogen-free diet, 10 wks for establishment; based on previous studies	Bilateral proximal tibia	Complete osteotomy	Plate and screws; 83% (30/36, n = 36), 6 rats had intra-operative fibula fracture	CT: achieved adequate fracture healing at 5 wks	35	Strengths: clinically relevant model; OVX rats are FDA-approved model; easy to handle, reproducible. Weaknesses: bilateral osteotomy has negative influence in weight-bearing and welfare concerns	Study of potential therapeutic agents, e.g. Black Cohosh supplementation. Black Cohosh supplementation did not have positive effects in severe osteoporosis
16	Komrakova et al[36]	2010	Rat	OVX on 3-mth-old SD-rat with soy-free diet, 8 wks for establishment; micro-CT	Bilateral proximal tibia	Complete osteotomy	Plate and screws; 92% (88/96, n = 96), 4 rats had intra-operative fibula fractures and 4 died throughout study	Histology: osseous bridging at day 23	35	Strengths: clinically relevant model; OVX rats are FDA-approved model; easy to handle, reproducible. Weaknesses: bilateral osteotomy has negative influence in weight-bearing and welfare concerns	Study of potential therapeutic agents, e.g. human PTH. PTH alone promotes bone healing in ovariectomized and sham rats when applied in the early stage of healing without having adverse effects
17	Kolios et al[25]	2010	Rat	OVX and fracture at same time on 3-mth-old SD-rats; new model on early phase of osteoporosis	Bilateral proximal tibia	Complete osteotomy	Plate and screws; 83% (30/36, n = 36), 5 rats had intra-operative fibula fracture, 1 had infection	CT: achieved adequate fracture healing at 5 wks	35	Strengths: clinically relevant model; able to study early phase of osteoporosis; OVX rats are FDA-approved model; easy to handle, reproducible. Weaknesses: bilateral osteotomy has negative influence in weight-bearing and welfare concerns	Study of therapeutic agents in early phase of osteoporosis, e.g. Black Cohosh and oestrogen. Oestrogen improved the biomechanical properties of callus in early osteoporosis
18	Stuermer et al[33]	2010	Rat	OVX on 3-mth-old SD-rats, 10 wks for establishment; based on previous studies	Bilateral proximal tibia	Complete osteotomy	Plates and screws; 73% (22/30, n = 30), 1 rat died from OVX and 7 had intra-operative fibula fracture	Histology: osseous bridging at 22 days	35	Strengths: clinically relevant model; OVX rats are FDA-approved model; easy to handle, reproducible. Weaknesses: bilateral osteotomy has negative influence in weight-bearing and welfare concerns	Study of potential therapeutic interventions, e.g. WBV. WBV alone improved cortical and callus densities, trabecular structures, blood supply, and oxidative metabolism in ovariectomized rats. WBV also increases muscle fibre size
19	Stuermer et al[10]	2010	Rat	OVX and fracture at same time on 3-mth-old SD-rats; new model on early phase of osteoporosis	Bilateral proximal tibia	Complete osteotomy	Plate and screws; 97% (29/30, n = 30), 1 rat had infection	X-ray: osteoporotic rats achieved adequate fracture healing at wk 5. Histology: unstructured bone in the beginning, followed by bony bridging after 25 days and increase in endosteal callus afterwards to 5 wks	35	Strengths: clinically relevant model; able to study early phase of osteoporosis; OVX rats are FDA-approved model; easy to handle, reproducible. Weaknesses: bilateral osteotomy has negative influence in weight-bearing and welfare concerns	Study of therapeutic agents in early phase of osteoporosis, e.g. oestrogen and raloxifene. Raloxifene and oestrogen both had supporting effects in fracture healing in rats in early phase of osteoporosis by improving callus structure and mechanical properties

OVX, ovariectomized; pQCT, peripheral quantitative computed tomography; HR, high resolution; FDA, the Food and Drug Administration; HA, hydroxyapatite; HA/col-1, hydroxyapatite/collagen composite 1; SD-rat, Sprague Dawley rat; CT, computed tomography; PTH, parathyroid hormone; WBV, whole body vibration; SR, strontium ranelate; BMD, bone mass density; DXA, dual-energy X-ray absorptiometry; SrCPC, strontium (II)-modified calcium phosphate cement; CPC, calcium phosphate cement; Scl-Ab, sclerostin antibody