INTRODUCTION:
Femoral head necrosis (FHN) is a common complication after high dose corticosteroid treatment. In the current study we investigated the possible role of vascular endothelial growth factor (VEGF) and its receptors VEGFR-2 in steroid induced femoral head necrosis and disordered bone remodeling by biochemical and immunohistochemical methods. Using cultivated human osteoblasts as a model, our aim was to investigate altered VEGF levels due to GC treatment, and steroid induced femoral head necrosis.

MATERIALS AND METHODS:
Femoral heads (n=6) were obtained from patients undergoing total hip arthroplasty (age 32-54) for steroid related late stage (ARCO IV) femoral head necrosis. Human osteoblasts were derived proliferating from Oligene (Berlin, Germany) and cultured under standard conditions. Cells were incubated for 24 and 72 hours with dexamethasone (100 µMol) and epidermal growth factor (EGF; TEBU, Offenbach, Germany), diluted to 10 ng/ml. Stimulation experiments were carried out in triplicates. Cell culture experiments were repeated for n=6 times. For histology and immunohistochemistry, tissue samples were fixed in 3% paraformaldehyde, embedded in paraffin, sectioned, dewaxed, and incubated for 15-20 min. Deparaffinisation was achieved by washing in xylene (twice for 5 min), followed by a graded series of ethanol (alkohol) solutions and incubation in phosphate buffered saline (PBS) for 10 min. Tissue sections were incubated for 30 min with normal serum (1:5 in Tris-buffered saline) of the species in which the primary antibody. Osteoblasts of the non-necrotic femoral head areas were clearly immunostained for VEGF and KDR expression within the intra- and pericellular matrix. Osteoblasts of necrotic bone areas could also be immunostained for VEGF in case of necrotic bone disease of the femur. By a range of different methodological approaches we have demonstrated a strong GC-associated reduction of VEGF expression in the necrotic area of the femoral head. GC, glucocorticoids.

RESULTS:
Histology confirmed long lasting stage femoral head necrosis (ARCO IV), and deformity of the femoral head. Immunohistochemistry was performed to evaluate the expression of VEGF in case of necrotic bone disease of the femur. Within all sections of necrotic femoral heads VEGF could be immunostained in the intra- and pericellular matrix of osteoblasts in the necrotic bone area. VEGF immunostaining could be abolished by pre-incubation of the primary antibody with recombinant VEGF or by omitting the primary antibody. Osteoblasts of the non-necrotic bone area were clearly less immunoreactive to VEGF antibodies. Osteoblasts of necrotic bone areas could also be immunostained for VEGF receptor-2 (KDR). According to the staining intensity, VEGF and KDR-expression was clearly upregulated in case of steroid related femoral head necrosis in necrotic bone areas. ELISA confirmed VEGF induction in necrotic bone areas of femoral heads (Figure 1). Compared to non-necrotic bone samples, VEGF expression increases from 100 pg/ml to 490 pg/ml. To test whether cultured primary osteoblasts express VEGF and its receptors and if they were suitable for stimulation experiments, RT-PCR examinations were performed. Osteoblasts express the splice variants VEGF212 and VEGF165. We also analyzed the VEGF-receptor expression by RT-PCR experiments for verification of the immunohistochemical data. We detected one band corresponding to the VEGF receptor-2 (555 kb).

DISCUSSION:
The current study demonstrates an increased expression of VEGF in osteoblasts from necrotic femoral heads and this may be connected with the process of revascularization and bone ingrowth into the necrotic area as the femoral heads were harvested in later stage osteonecrosis. Ohzono et al. examined human femoral heads at Ficat’s stages 2 to 4 histopathologically, and by microangiography (1). They described that reparative arterioles grew on the trunk of the intracapital nutrient arteries. They also described an avascular zone directly subchondrally, an underlying reparative vascular zone, and a normal vascular zone. In the reparative vascular zone, they described an advancing ingrowth of reparative vessels from stage 2 to 4. These were described as sparse in stage 2, a fine network in stage 3, and a dense network in stage 4. The new results of increased VEGF expression in the necrotic femoral heads of the present study well correlate with the findings of Ohzono et al. Vascular endothelial growth factor is increased, and stimulates the ingrowth of reparative vessels into the necrotic femoral head. On the other hand, the decrease in VEGF detected in the in-vitro part of the present study in osteoblasts incubated with GC may support the development of initial osteonecrosis. This may be an additional pathogenetic factor in the early stage of femoral head necrosis. GC impairs vessel ingrowth into the endarterial bed of the femoral head (18), and makes it more vulnerable to ischemia. Vessels have been shown to be essential to osteogenesis (3). In our study of GC induced femoral head necrosis and GC induced VEGF decrease in osteoblasts we indeed observed strong significant hints for participation of VEGF in femoral head necrosis. By a range of different methodological approaches we have demonstrated that VEGF is expressed by osteoblasts and is affected by dexamethasone. These data strongly suggest that VEGF plays an important role in the initial stage of femoral head necrosis. Since VEGF is a potent angiogenic peptide, it is likely to be responsible for the neovascularisation observed in remodeled bone. Furthermore, we have demonstrated a strong GC-associated reduction of VEGF expression in human osteoblasts. The observed increase of VEGF in necrotic bone areas of femoral heads seems to stimulate the ingrowth of reparative arterioles into the necrotic femoral head. REFERENCES: