munohistochemical localization of LEPR and study the effect of leptin on the expression of dentin sialophosphoprotein (DSPP) by human pulp cells.

Materials and Methods
Twenty-five dental pulp samples were obtained from freshly caries- and restoration-free extracted human third molars. The pulp samples were processed and mineralization produced by odontoblasts in response to leptin was determined analyzing the expression of DSPP by immunoblot and by real time PCR (qRT-PCR). LEPR localization was examined by immunohistochemistry using anti-human LEPR monoclonal antibody.

Results
The immunoreactivity for antibodies anti-LEPR was localized in the odontoblastic layer and the predentine. Leptin dose-dependently stimulated dentin sialophosphoprotein expression in human dental pulp. Western blot analysis of leptin-stimulated human dental pulp samples revealed the presence of a protein with an apparent molecular weight of approximately 100 kDa, which corresponds to the estimated molecular weight of DSPP. The expression of DSPP mRNA was confirmed by qRT-PCR analysis, and the size of the amplified fragments was confirmed by agarose gel electrophoresis.

Conclusions
For the first time it has been demonstrated that human odontoblasts express the leptin receptor (LEPR), and the binding of leptin to LEPR results in DSPP production by odontoblasts. These findings suggest that leptin plays a role in the defensive response pulp and dentinogenesis.

- Oral Presentation 48
TITLE: Activation of PKB Pathway signaling by leptin in human dental pulp


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Objectives
Leptin, initially described as an adipocyte-derived hormone to regulate weight control, as well as its receptor (LEPR), are expressed in human dental pulp. Both leptin and LEPR are up-regulated during pulp experimental inflammation. This study aims to assess if leptin signal transduction in human dental pulp involves PKB phosphorylation.

Materials and Methods
Fifteen dental pulp samples were obtained from freshly caries- and restoration-free extracted human third molars. Pulp samples were processed and leptin signaling was determined analyzing PKB phosphorylation by immunoblot. To measure activation of PI3K pathway in human dental pulp in response to human leptin, the activation of the central kinase of this pathway, i.e. PKB, was measured using antibodies that specifically recognize the phosphorylated form of PKB (P-PKB). Anti-β-tubulin antibodies were used for the control of the immunoblot.

Results
Leptin stimulated PKB phosphorylation. The phosphorylated band corresponded with an apparent molecular mass of about 60 kDa, which corresponds to the estimated molecular weight of P-PKB. An increase in phosphorylation was observed at 0.1 nM leptin, maintaining the effect at 1 and 10 nM leptin. The relative amount of PKB in stimulated pulps was significantly higher than in unstimulated pulps (p < 0.05).

Conclusions
PKB is involved in leptin signalling pathways in human dental pulp.

- Oral Presentation 49
TITLE: Root perforations in central incisors: 12 years of evolution

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Introduction
In the endodontics field, iatrogenesis is a common and very complex issue, we had the chance to perform a retreatment in a patient with two root perforations performed while trying to access the pulp chamber of 1.1 and 1.2, due to lack of direction in the access opening.

Case report
A 65 years-old patient was referred to our clinic to retreat endodontically both central incisors after a previous failed attempt to in which the deviation of the axis to access cavity did a root perforation iatrogenically in