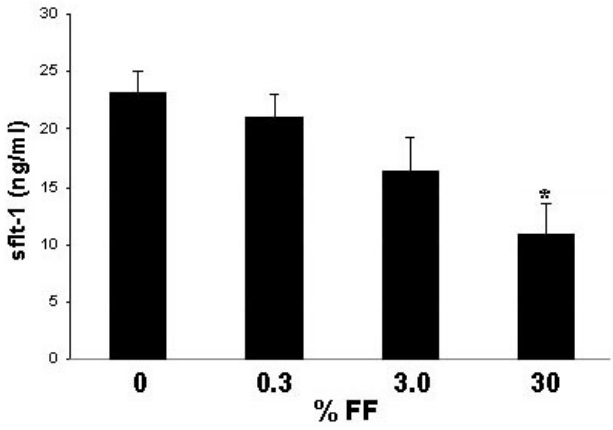

Figure 3
Amount of sFlt-1 in the culture supernatant of HUVECs after 4 days of incubation with medium containing different concentrations of human follicular fluid. Amount of sFlt-1 decreases with increasing concentrations of follicular fluid showing a significant inhibition of sFlt-1 production at a concentration of 30%. * = $p < 0.05$.

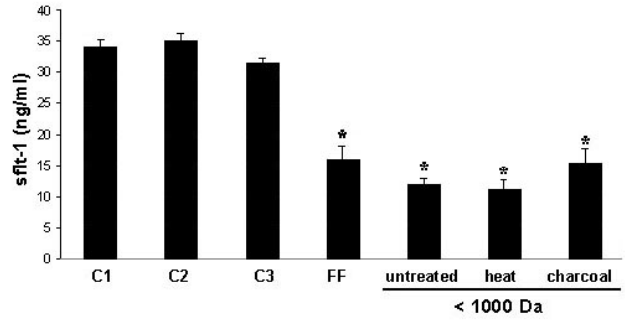

Figure 4
Quantification of sFlt-1 in endothelial cell culture supernatant. Neither heat inactivation of culture medium containing FCS (C2) nor absence of FCS (C3) has a measurable effect on sFlt-1 production of endothelial cells compared to incubation with untreated control medium containing FCS (C1). The significant inhibitory effect of untreated human follicular fluid (FF) on sFlt-1 production is maintained after ultra filtration leaving only molecules smaller than 1000 Dalton. This inhibition is not prevented neither by heat inactivation nor by charcoal treatment of the follicular fluid-flow through. All follicular fluids have been added to the culture medium at a concentration of 30%. * = $p < 0.05$

3', β-actin reverse 5'-TACGGCCAGAGGCGTACAGGGATAG-3' (product size 214 bp). Amplifications were run in 50 µl volume using BioTherm Taq polymerase (Genecraft, Muenster, Germany) for 35 amplification cycles of 30 sec denaturation at 94°C, 45 sec annealing at 62°C and 30 sec elongation at 72°C. The PCR amplification was followed by a 10 minute final extension at 72°C. The conditions were chosen so that the sflt-1 cDNA as well as the control β-actin cDNA were in the exponential phase of amplification and did not reach a plateau at the end of the amplification protocol. The generated PCR amplification products were electrophoresed on a 2% agarose gel and detected by ethidiumbromide staining. PCR products were normalized to β-actin by densitometric analysis using a Gel imager (Intas, Goettingen, Germany) and were relatively quantified (Gelscan Professional V4.0).

Statistical analysis

Statistical analysis was performed using the non-parametric Mann-Whitney test. The level of significance was set at $p < 0.05$.

Results

Regulation of endothelial cell proliferation and sFlt-1 secretion by follicular fluid

Incubation of HUVECs with culture medium containing 30% follicular fluid led to a significant increase in cell number compared to controls on day 3 and 4 of culturing (Fig. 1). Analyzing secretion of sFlt-1 by endothelial cells, HUVECs secreted 14.63 ± 1.36 ng sFlt-1 per ml into the culture medium during 24 hours of monolayer culture (Fig. 2). After 3 days of culture the amount of secreted sFlt-1 accumulated to 37.2 ± 5.2 ng/ml. This increase in sFlt-1 -1 was inhibited by the presence of 30% follicular fluid in the culture medium (Fig. 2). The inhibition of sFlt-1 production was proven to show a dose-effect as it was dependent on the concentration of follicular fluid in the culture medium. The amount of sFlt-1 decreased with increasing concentration of follicular fluid, showing a significant inhibition of sFlt-1 production at a concentration of 30% (Fig. 3).

To exclude an unspecific role of FCS on sFlt-1 production in HUVECs, culture medium containing FCS had been either untreated, heat inactivated or FCS was omitted. There was no measurable effect of either of these controls on sFlt-1 production of endothelial cells (Fig. 4). To analyze the follicular fluid in regard to factors possibly responsible for sFlt-1 regulation, follicular fluid has been partially purified by ultrafiltration. The resulting flow through containing only molecules smaller than 1000 Dalton still significantly inhibited sFlt-1 secretion by endothelial cells. Moreover, this effect could not be prevented by exposure of the flow through to heat or charcoal treatment (Fig. 4).