

## The deficits of matrigel plug assay

Sir,

We read with great interest the article by Tahergorabi *et al.* on "Ghrelin does not modulate angiogenesis in matrigel plug (BD Biosciences) in normal and diet-induced obese mice" published in "Journal of Research in Medical Sciences: The Official Journal of Isfahan University of Medical Sciences Caspian Journal of Internal Medicine."<sup>[1]</sup> Tahergorabi *et al.* reported some interesting results based mostly on statistical associations that, there was a strong positive correlation between the number of CD31-positive cells and serum leptin concentration ( $r = 0.91$ ). However, we think that some points should be discussed.

Angiogenesis is a very complex process containing many angiogenic and antiangiogenic factors. The subcutaneous matrigel plug assay in mice is a method of choice for the *in vivo* evaluation of pro- and antiangiogenic molecules. Matrigel containing test cells or substances is injected subcutaneously, where it solidifies to form a plug. This plug can be recovered after 7-21 days in the animal and examined histologically to determine the extent to which blood vessels have entered it.<sup>[2-4]</sup>

In this study, matrigel plugs were removed after 10-day from administration and at the same time systemic serum leptin levels was measured and a strong positive correlation between the number of CD31-positive cells in the matrigel plug with leptin and serum leptin levels ( $r = 0.91$ ;  $P < 0.05$ ) was reported. The comparison of systemic serum leptin levels and CD31-positive

cells in the matrigel plug (local administration) is not suitable for statically. If the leptin levels were measured before and after matrigel administration, it should have been more meaningful. Because 10-day application of matrigel plug with leptin can't affect the systemic leptin results. In addition, as shown in recent studies, high fat diet-induced obesity causes increasing in leptin levels.<sup>[5]</sup>

In conclusion, it would be appreciated if the authors could present some more data adjusted for major confounders.

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