

***In vitro* proliferation of uterine fibroids is related with vitamin D receptor and patient's age.**

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Keywords: Uterine fibroids (UF), Vitamin D (VitD), Vitamin D Receptor (VDR), Age, *In vitro* Proliferation rates.

Study question (25 words): Could Vitamin D be a potential uterine fibroid treatment for women of all ages?

Summary answer (25 words): Vitamin D, acting via its receptor, is a potential treatment for uterine fibroids, which occur in women at reproductive ages.

What is known already (100 words): Uterine fibroids (UF) (also known as leiomyomas or myomas) are benign tumours occurring in an estimated 20%-40% of women during their reproductive years. UF affect a patient's quality of life, as well as her fertility and obstetrical outcomes. Recent studies have proposed Vitamin D (VitD) as an effective treatment for UF. Diverse functions for VitD have been confirmed by the presence of vitamin D receptor (VDR) in a wide range of human tissues, including the myometrium of the human uterus, but its specific role in UF remains unclear.

Study design (75 words): This study was approved by the Clinical Research Ethics Committee at the Hospital Universitario y Politécnico La Fe de Valencia (Spain) (2014/0691). Our pilot study was carried out in the Hospital Universitario y Politécnico La Fe de Valencia and Fundación Instituto Valenciano de Infertilidad (FIVI). Intramural UF were collected from patients undergoing surgery due to UF pathology (n=14) without any previous treatment. All participants provided written informed consent.

Participants/materials, setting, methods (75 words): UF tissue (n=14) was analysed by Western blot to determine the presence of Vitamin D receptor (VDR) (Cell Signaling antibody) protein. Subsequently, UF tissue was disaggregated with collagenase-II (Sigma-Aldrich) and cells were cultured in DMEM-F12 medium. *In vitro* cell proliferation assays were performed by flow cytometry using propidium iodide. Data were normally distributed; parametric linear regression and Student's t-test were conducted with R commander software.

Main results (100 words): We corroborated the statistically significant negative correlation between *in vitro* proliferation of UF cells and patient age (ranging from 22 to 52 years, p-value = 0.028). Additionally, cells from UF with VDR showed greater cell growth compared with cells without VDR (p = 0.008). Surprisingly, we observed a trend in which the VDR was absent in patients ranging from 44-52 years old (p = 0.07). These findings were supported by the statistically significant correlation between *in vitro* proliferation, presence of VDR, and patient age (p = 0.049), suggesting a strong interaction among the three.

Limitations (50 words): Limitations of this pilot study include the small sample size.

Wider implications of the finding (50 words): Active uterine fibroid cells appear during the reproductive years and regress around pre-menopause. These active cells exhibit an *in vitro*

proliferation rate that correlates with the presence of vitamin D receptor and the patient's age. These findings provide a foundation for exploring vitamin D treatment in the clinical setting.

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