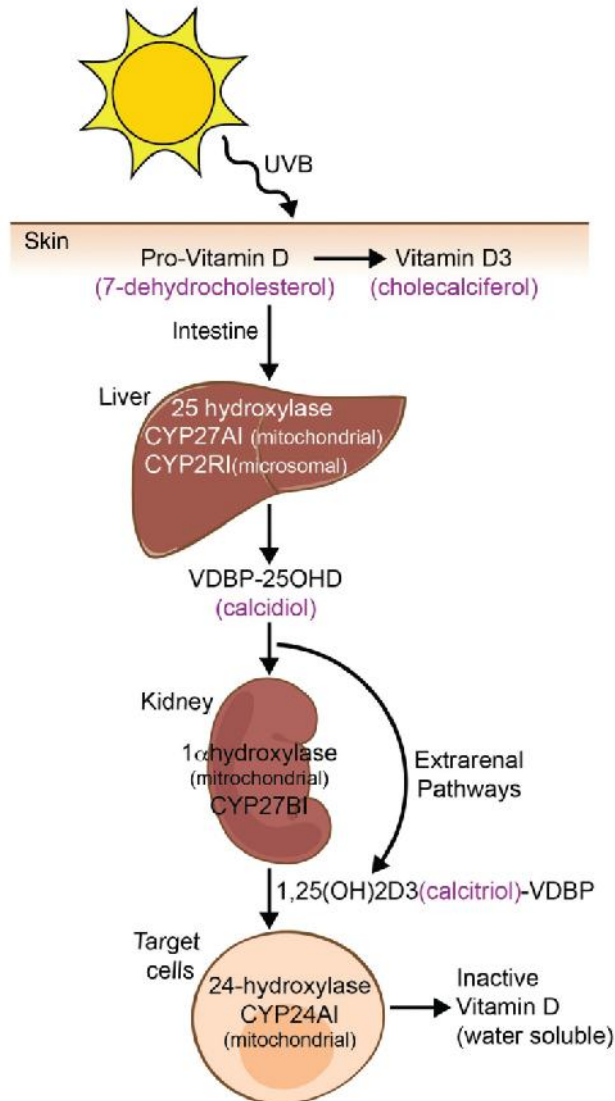


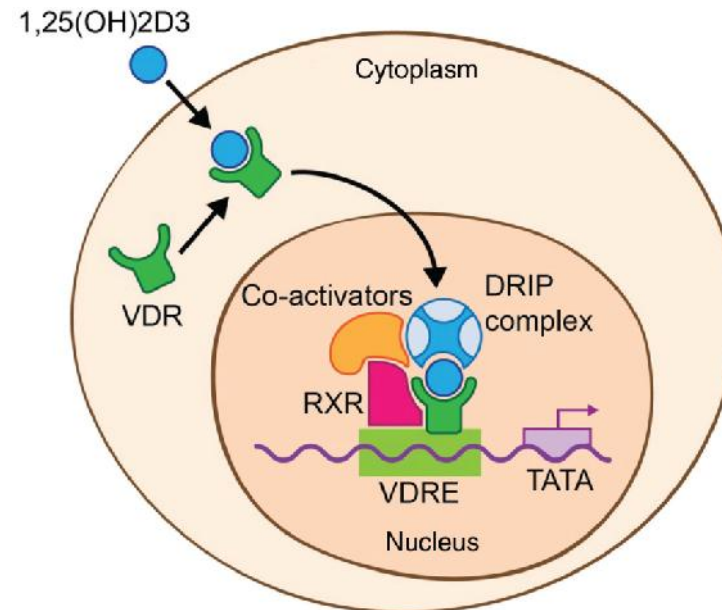
Vitamin D and Human Reproduction: Past, Present and Future

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Professor of Immunology

The major source of vitamin D is the skin



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Pandemic of vitamin D deficiency

The data collected by the National Health and Nutrition Examination Surveys within North America document a 4-fold increase in the prevalence of vitamin D deficiency over the past 10–15 years.

Populations with the greatest physiological needs for vitamin D, such as pregnant women, neonates, children and adolescents are also at highest risk for vitamin D deficiency.

Suboptimal dietary vitamin D intake

Increasing environmental pollution

A shift in lifestyle with consequent reduced sun exposure

Obesity

Increased use of sunscreen

Vitamin D and reproduction

1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃ or calcitriol) is the major regulator of **calcium and phosphate homeostasis**.

Apart from the well known effects of vitamin D on maintaining calcium homeostasis and promoting bone mineralization, there is some evidence suggesting that **vitamin D also modulates human reproductive processes**.

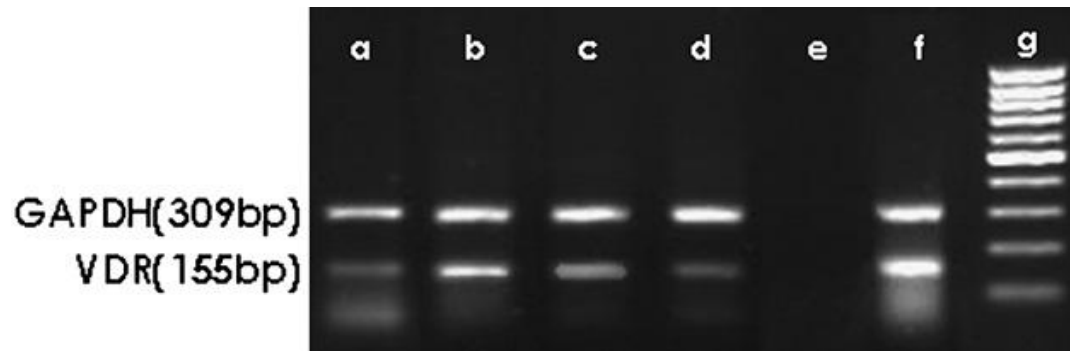
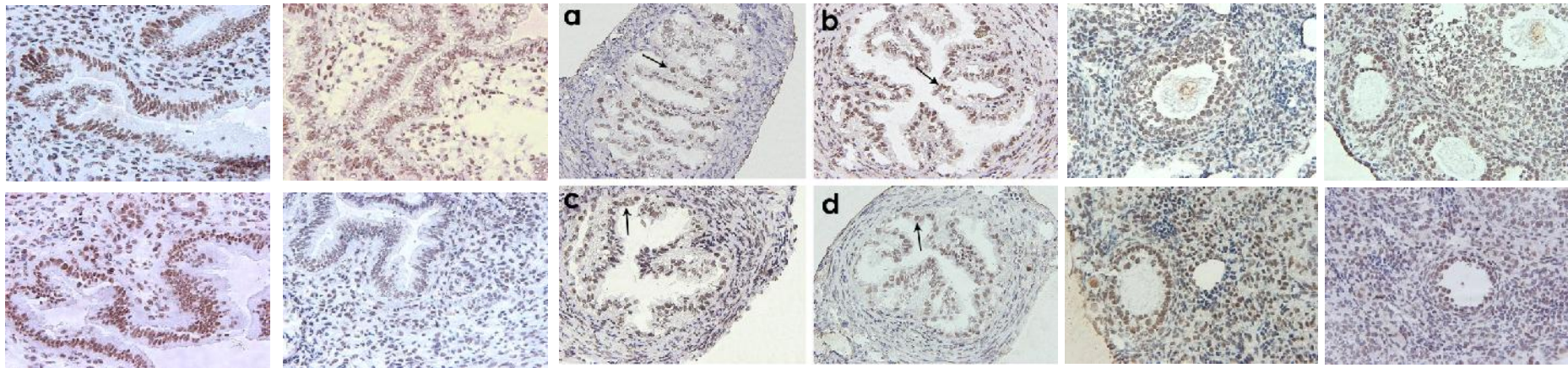
During pregnancy, maternal circulating levels of vitamin D3 are increased.

Expression of VDR by epithelial and stromal cells in mouse and human endometrium and its increased levels in pregnancy imply the importance of vitamin D3 in maintenance of normal pregnancy.

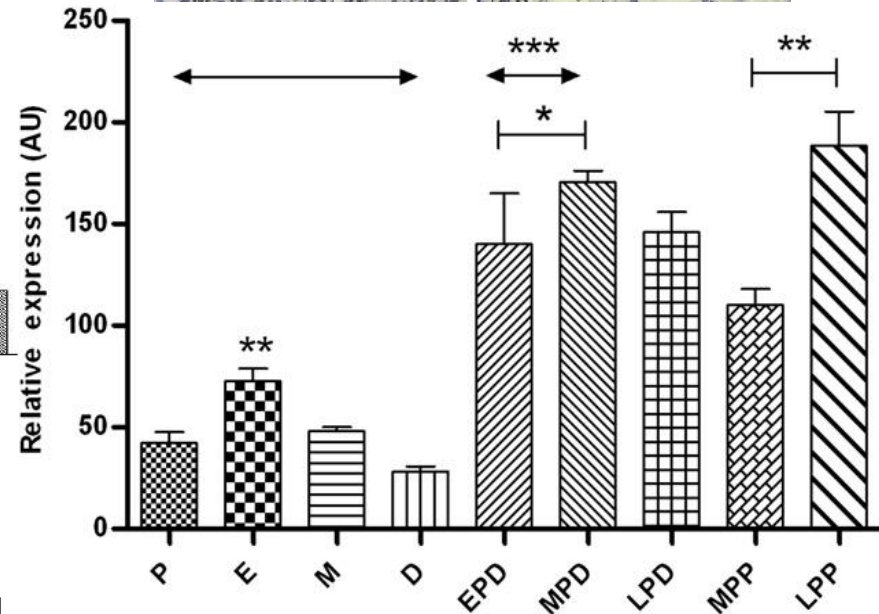
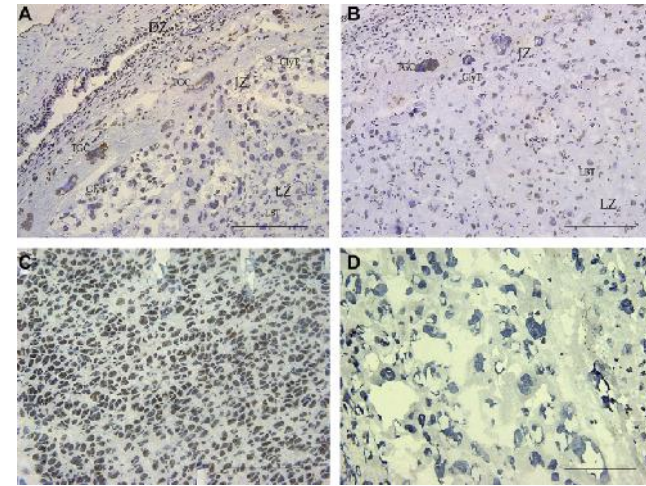
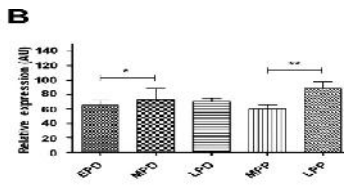
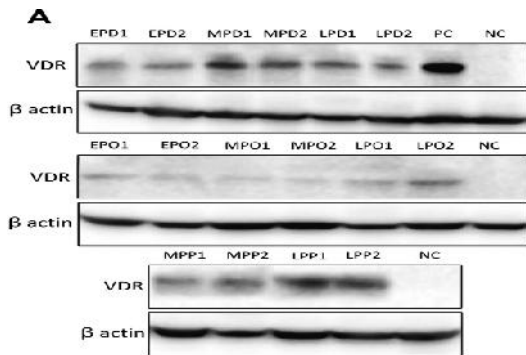
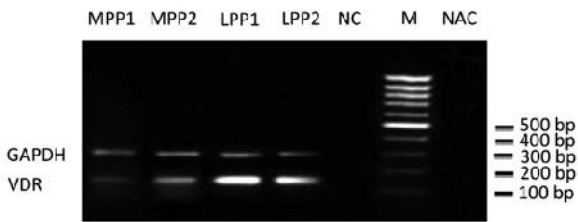
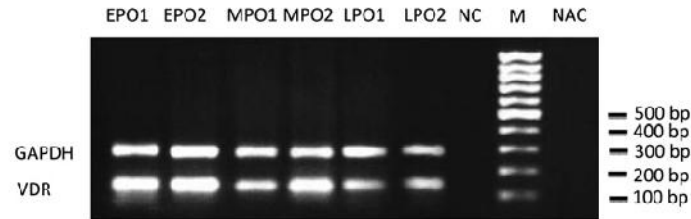
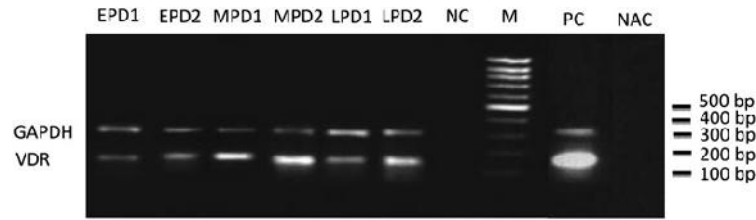
This hormone participates in endometrial decidualization, increases the abundance of HOXA10 (an essential element for implantation), and has important regulatory functions in placental development.

Recently, an association between vitamin D and uterine receptivity has been reported. For example, vitamin D could increase endometrial thickness in infertile women with polycystic ovary syndrome undergoing intrauterine insemination.

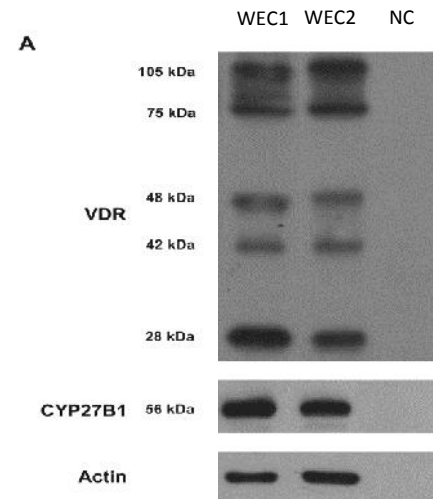
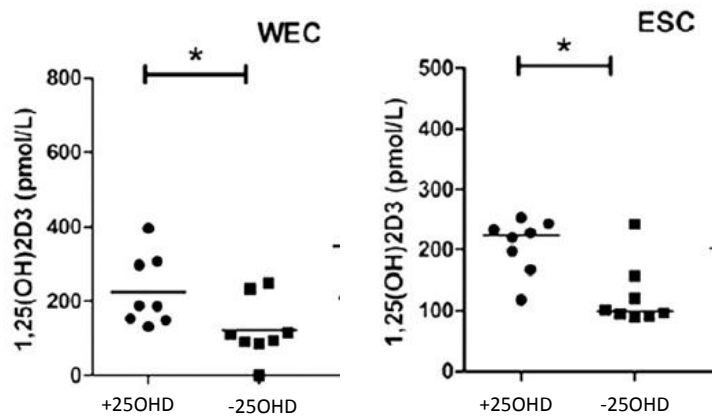
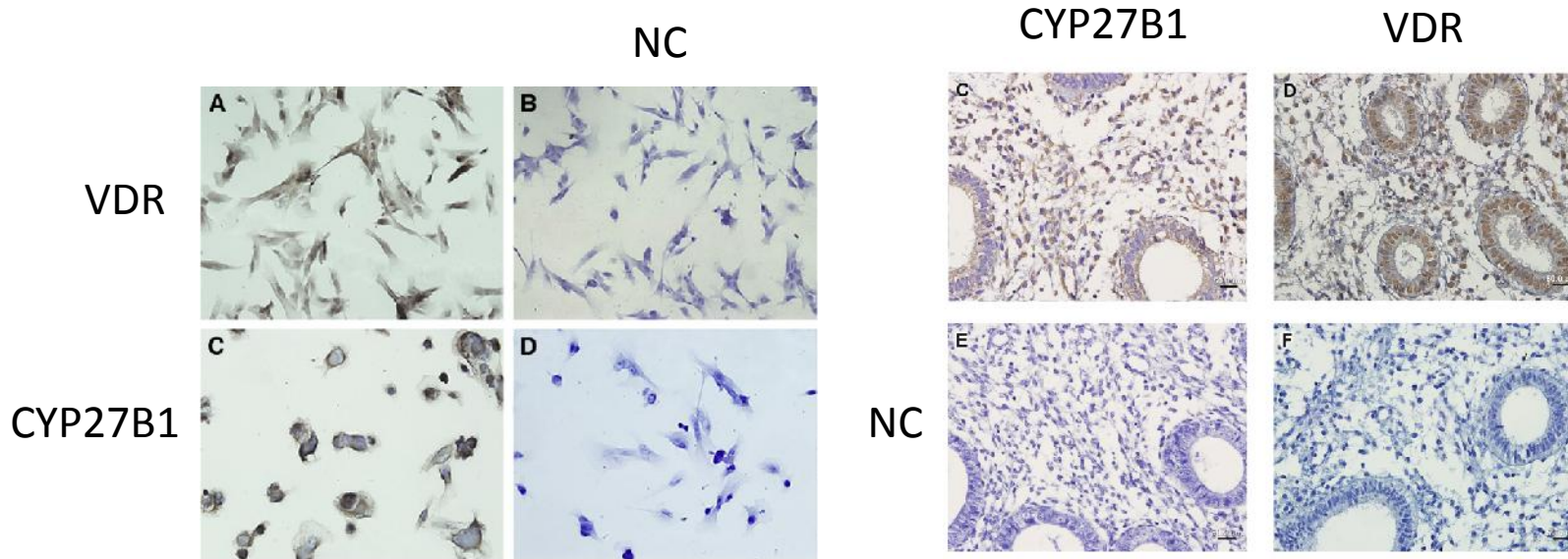
VDR is expressed in the reproductive organs of cycling mice



VDR is expressed in reproductive tissues throughout the pregnancy



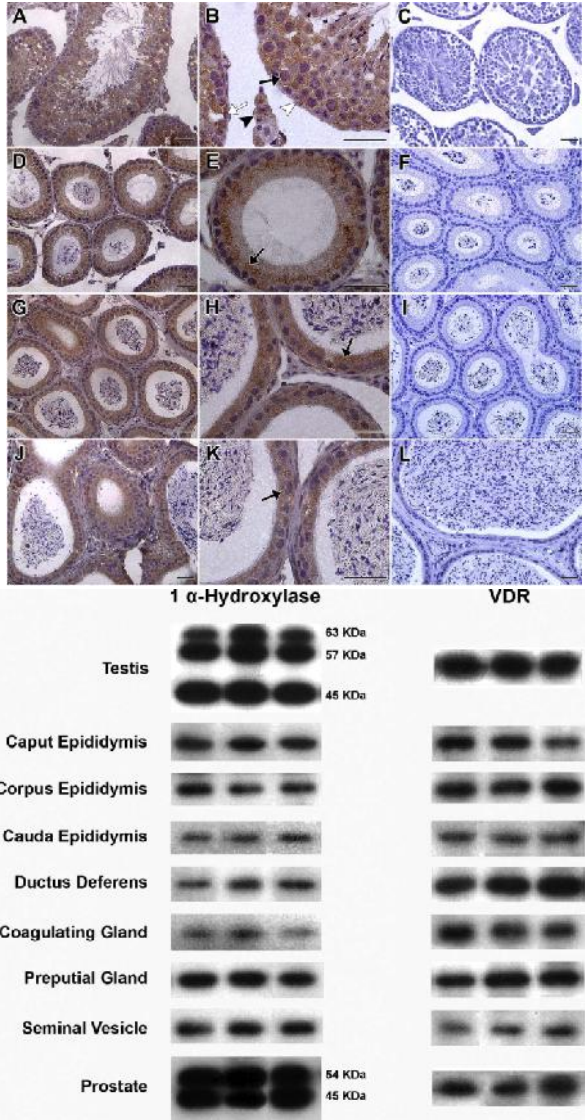
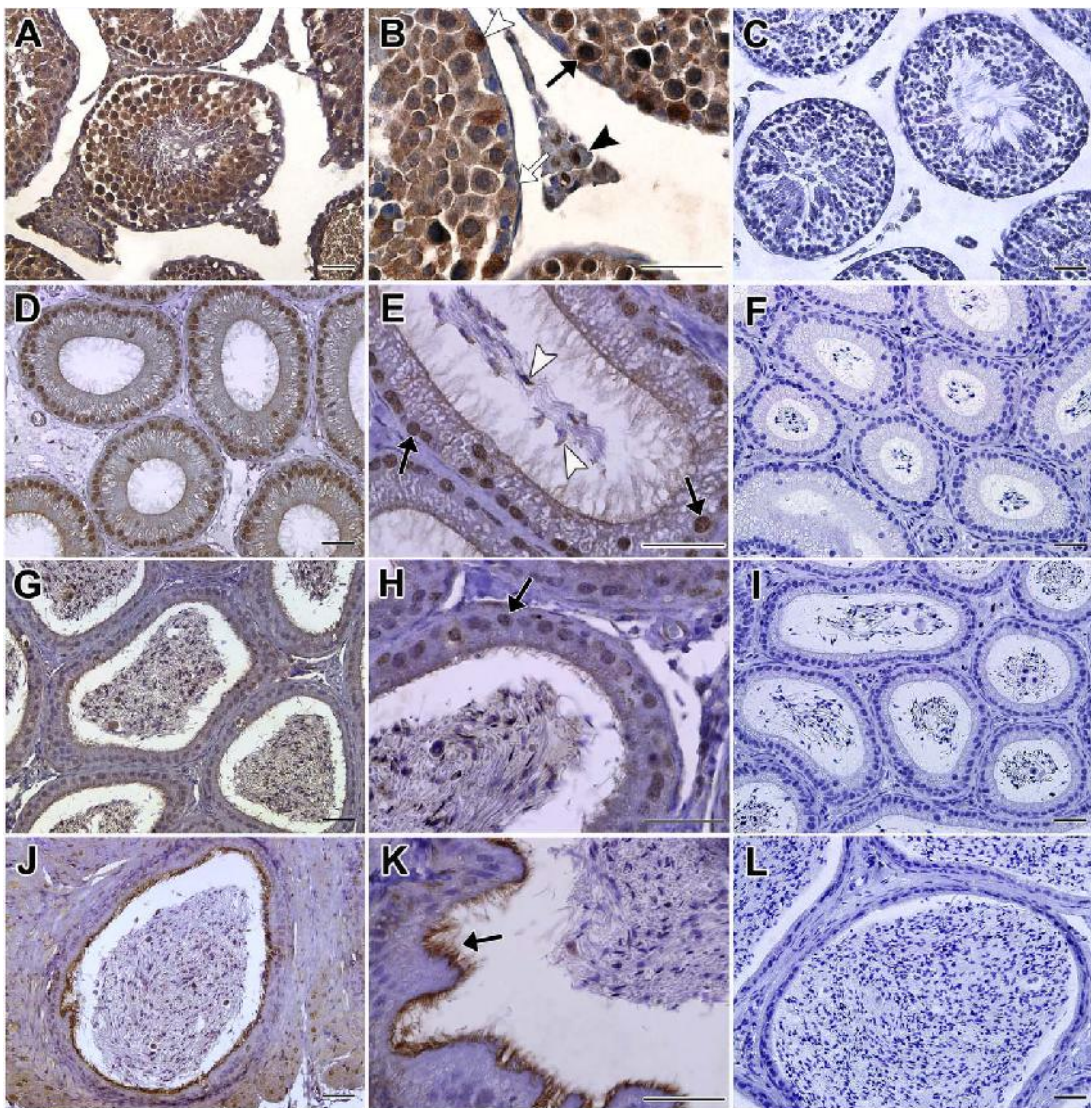
Human endometrial cells synthesize 1,25(OH)₂ D₃



Tavakoli et al. Fertil Steril. 2011 ;96(3):751-7

Tavakoli et al. Mol Reprod Dev. 2015;82(5):356-64

VDR and enzymes that metabolize vitamin D are widely expressed in male reproductive tract



vitamin D and ovarian reserve markers

According to available evidences, a relationship exists between **vitamin D and ovarian reserve markers**.

A wide range of **seasonal variations exist in AMH levels**. This variation is 18% lower than average during the winter season, where vitamin D levels are too low.

Vitamin D supplementations could prevent seasonal changes in both vitamin D and AMH levels.

A cross-sectional study that included 388 premenopausal women with regular menstrual cycles found a **positive association between serum vitamin D and AMH levels**.

Interestingly, in 1430 premenopausal women 30-42 years of age, **a negative correlation was found between serum vitamin D levels and urinary FSH levels**, which suggest that low vitamin D levels might influence ovarian reserve and predispose women toward earlier menopause.

Vitamin D, Infertility, and In Vitro Fertilization (IVF)

VDR is present and differentially expressed in murine **endometrium and ovary throughout the estrous cycle** whereas knockout experiments have shown that **VDR null mice** experience **uterine hypoplasia and impaired folliculogenesis**.

Expression of **1-alpha-hydroxylase** is upregulated in the human endometrial stromal cells of early **pregnant versus cycling endometria**.

However, in vivo data supporting a role for vitamin D in female fertility in general and embryo implantation in particular are not robust.

A recent retrospective study postulated that **vitamin deficiency may negatively affect pregnancy rates** with an effect mediated through the endometrium.

Up to date, only a few cohort studies have attempted to examine the role of vitamin D levels in infertile patients.

Results from these studies are **strongly contradictory**, with some findings showing that maternal vitamin D deficiency is associated with lower pregnancy rates and others demonstrating that vitamin D deficiency does not affect the final reproductive outcome.

Promising results have been reported about the **relationship between serum or follicular fluid levels of vitamin D and IVF outcomes**, specifically in terms of clinical pregnancy rates (CPR).

Women who achieved clinical pregnancy had significantly higher 25(OH)D levels in their serum and follicular fluid compared to those who did not become pregnant.

Each ng/ml increase in follicular fluid 25(OH)D enhanced the chance of achieving clinical pregnancy by 6%.

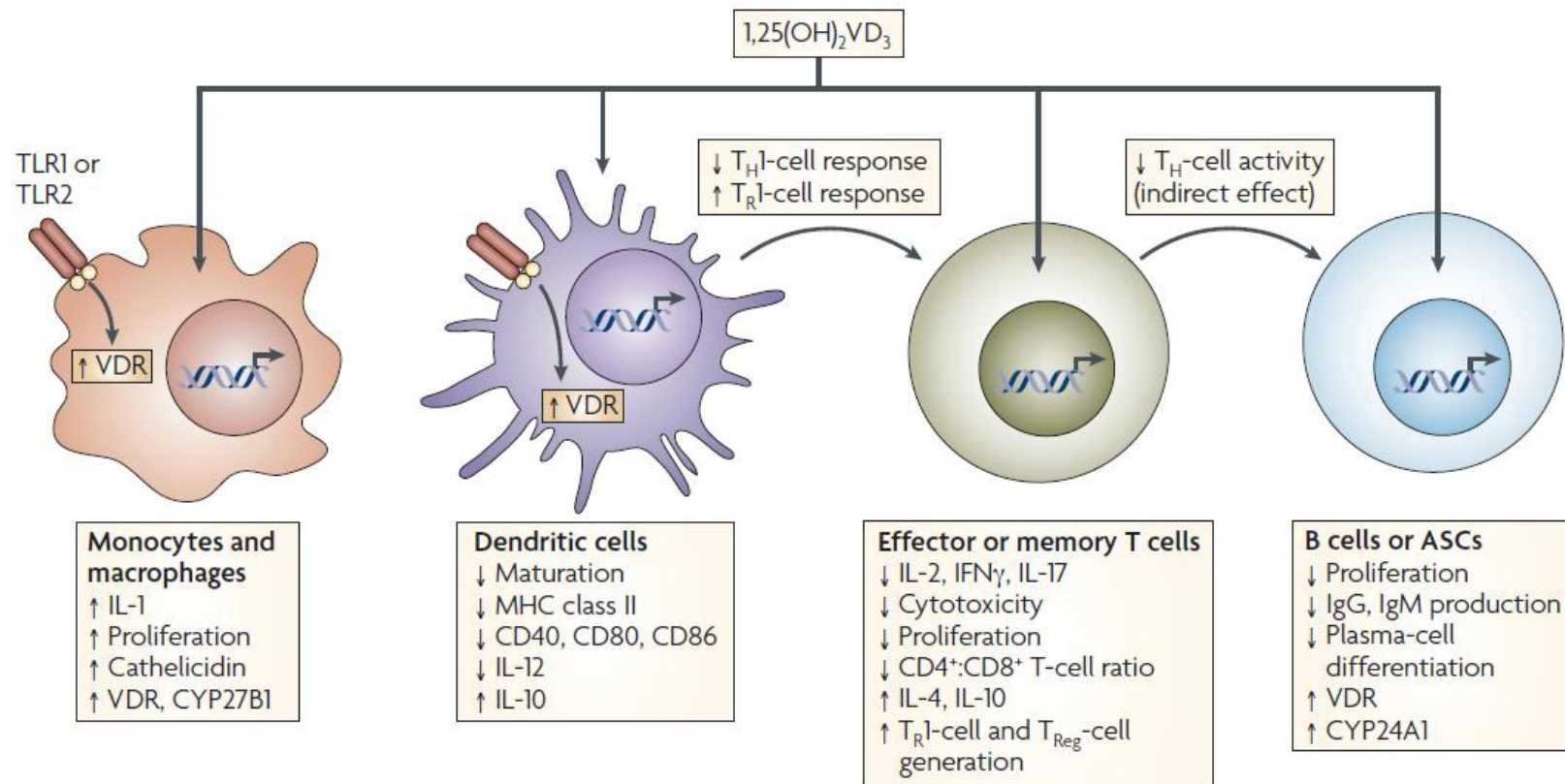
Other studies, however, **did not confirm** these findings.

Some researchers showed that **higher levels of 25(OH)D in follicular fluid negatively affected embryo quality** and led to poorer IVF outcome.

Relationship between vitamin D and IVF success **may be under the influence of the patient's ethnicity**. Vitamin D status showed a positive association with CPR among non-Hispanic white patients, but not among Asians.

Vitamin D and RPL

Mechanisms of vitamin D immunomodulation



Vitamin D deficiency may be a risk factor for recurrent pregnancy losses by increasing cellular immunity and autoimmunity

A retrospective cross-sectional study of 133 women with RPL who were enrolled in a 2-year period:

Higher prevalence of APA , ANA, anti-ssDNA and anti-TPO was significantly higher in VDlow than those of VDnl.

Peripheral blood CD19+ B and CD56+NK cell levels and were significantly higher in VDlow when compared with those of VDnl.

Reduction (%) of NK cytotoxicity by IgG was significantly lower in VDlow than those of VDnl.

Vitamin D3 significantly:

- ↓ NK cytotoxicity assay
- ↓ TNF- α /IL-10 expressing CD3+/4+ cell ratios
- ↓ INF-g/IL-10 expressing CD3+/4+ cell ratio
- ↓ IFN-g and TNF- α secretion from NK cells
- ↑ IL-10, IL-1 β , VEGF and GM-CSF from NK cells

Vitamin D insufficiency is associated with increased risk of first trimester miscarriage

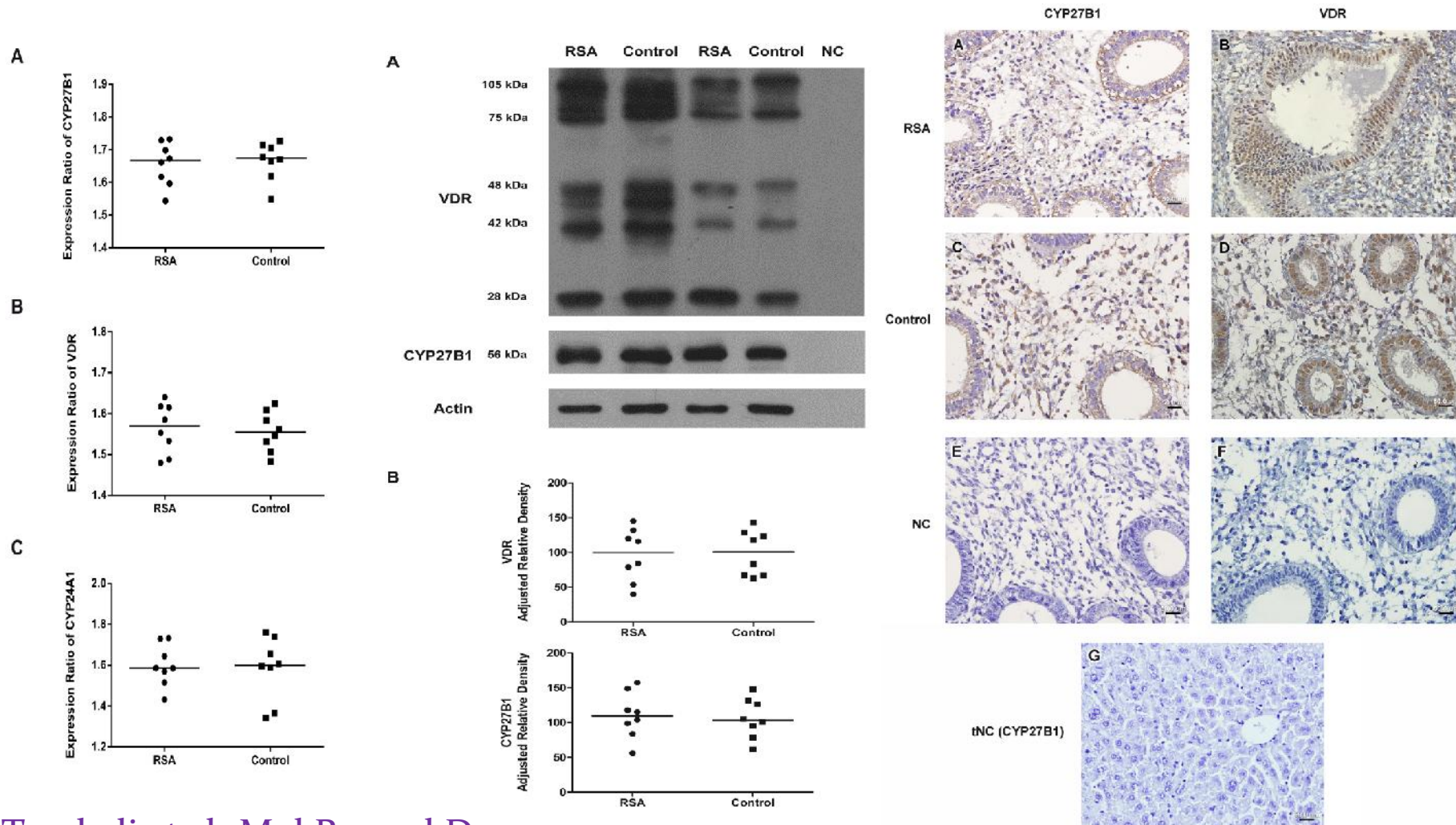
In a prospective cohort study of 1683 pregnant women before gestational week 22:

The adjusted hazard of first-trimester miscarriage was lower with higher 25(OH)D concentrations.

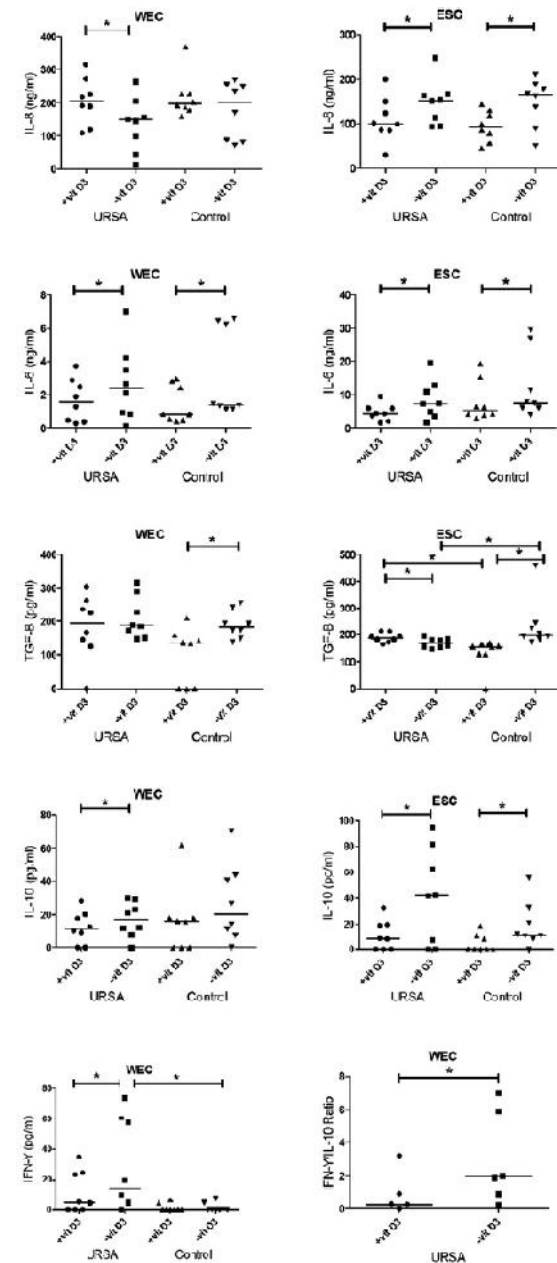
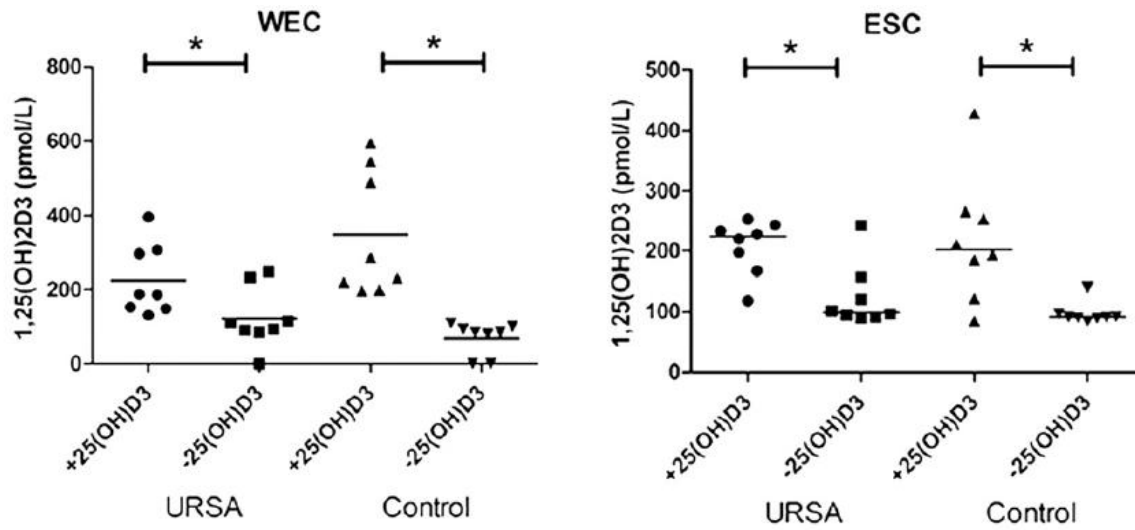
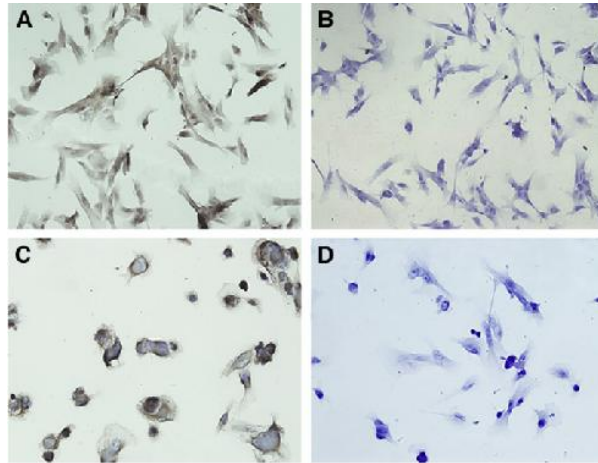
Concentrations of 25(OH)D ≤ 50 nmol/L were associated with a 2-fold increased adjusted HR for miscarriage.

Concentrations of 25(OH)D were not associated with an increased risk of second-trimester miscarriage.

Comparable levels of VDR, CYP27B1 and CYP24A1 in URSA and control Women



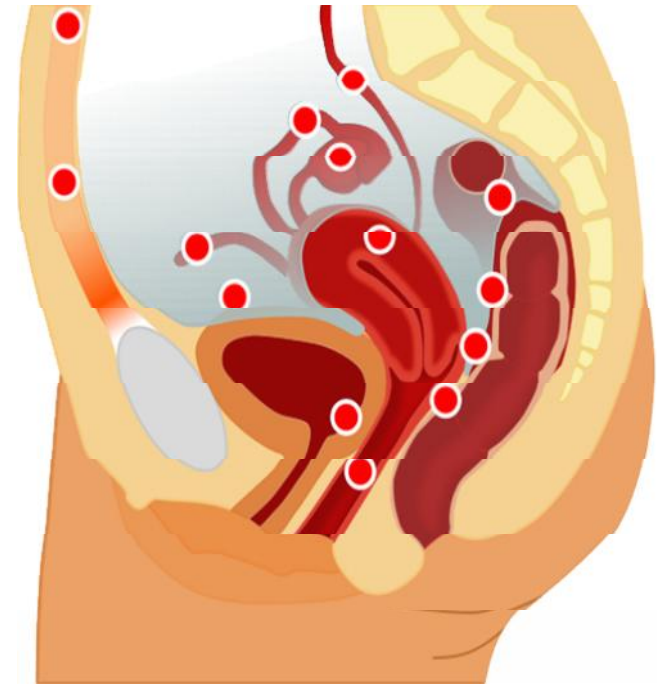
Comparable effect of vitamin D3 In URSA and control women



1,25-dihydroxy vitamin D₃ modulates endometriosis-related features of human endometriotic stromal cells

Endometriosis

- * Growth of endometrial glands and stroma at extra-uterine sites.
- * Leading cause of disability, responsible for dysmenorrhea, pelvic pain and subfertility
- * Affects about **3–10% of the female population** in the reproductive age, and up to **40–80% of women complaining of pelvic pain and or infertility.**
- * Increased risk of developing ovarian cancer

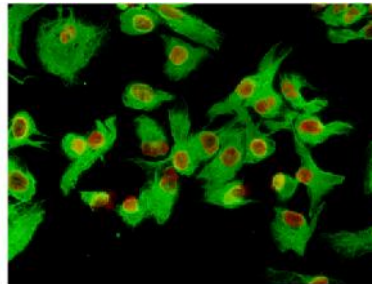


Endometriosis and vitamin D₃

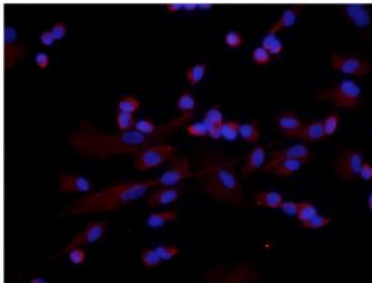
- ✱ Pleiotropic immunomodulatory effects in different pathologies mediated by chronic inflammatory responses.
- ✱ Lower weight, histologic score of lesions in a mouse model of endometriosis.
- ✱ Reduces adhesion capacity of mouse endometrial cells to collagen, and decreases peritoneal inflammation, macrophage recruitment and inflammatory cytokine secretion.

Immunophenotyping of EESCs, EuESCs, CESC, E-MenSCs and NE-MenSCs

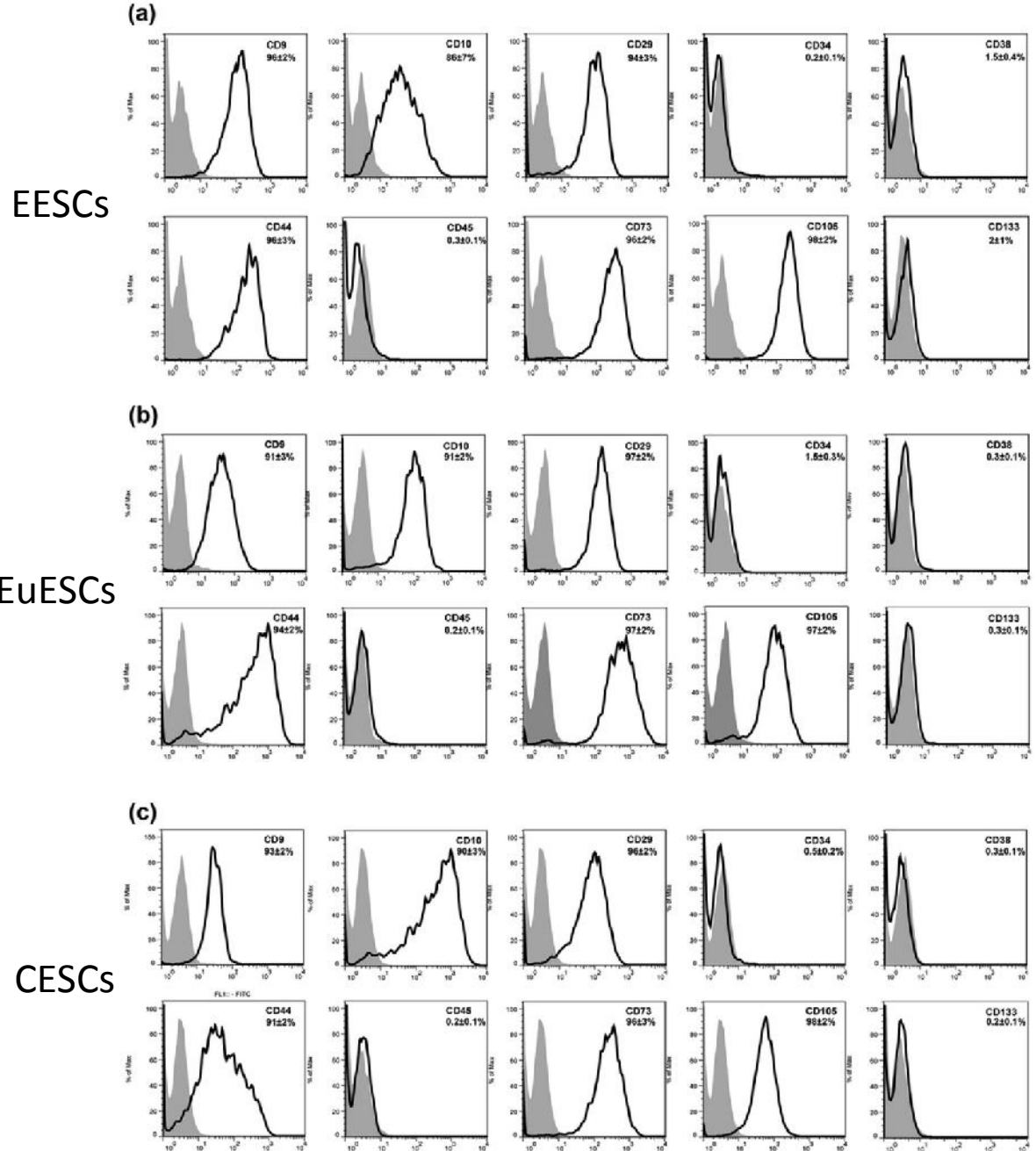
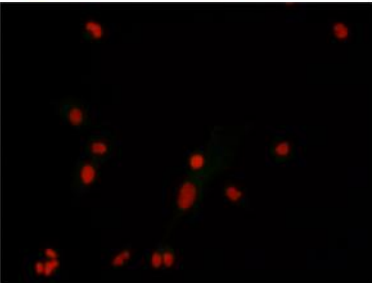
Vimentin



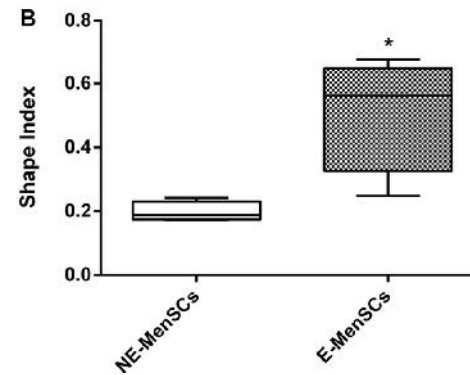
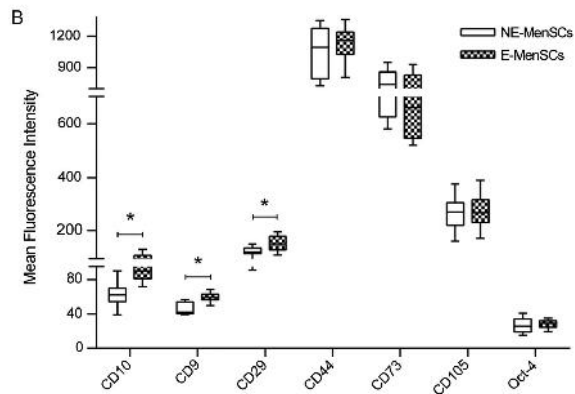
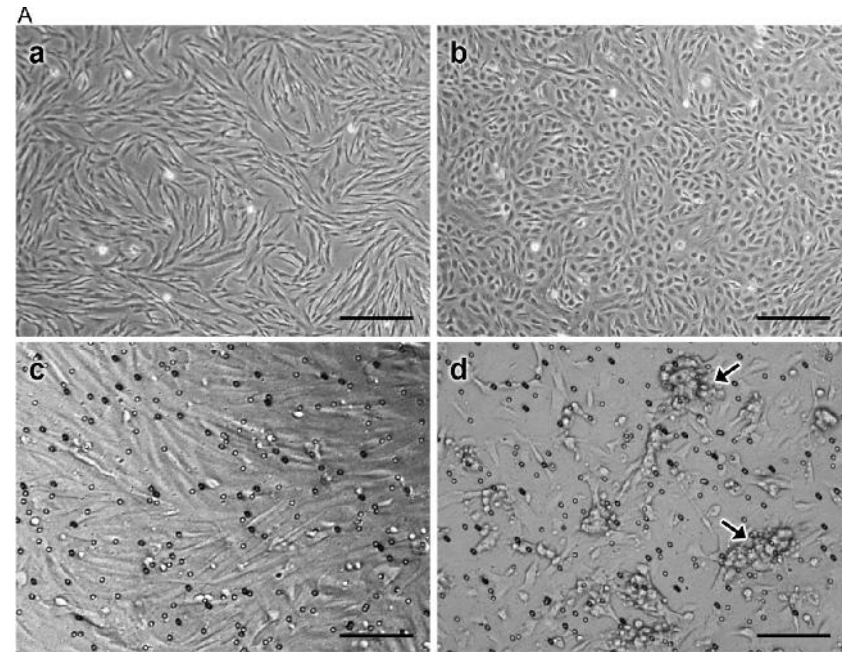
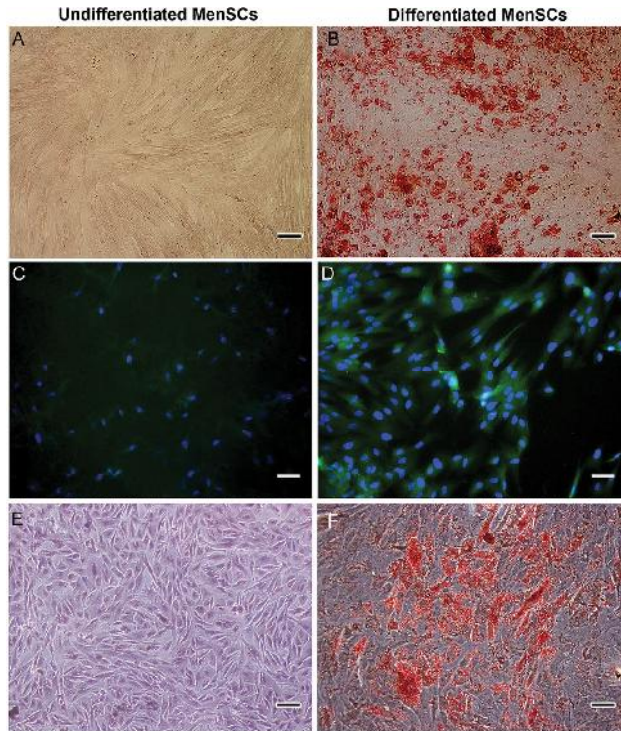
Nestin



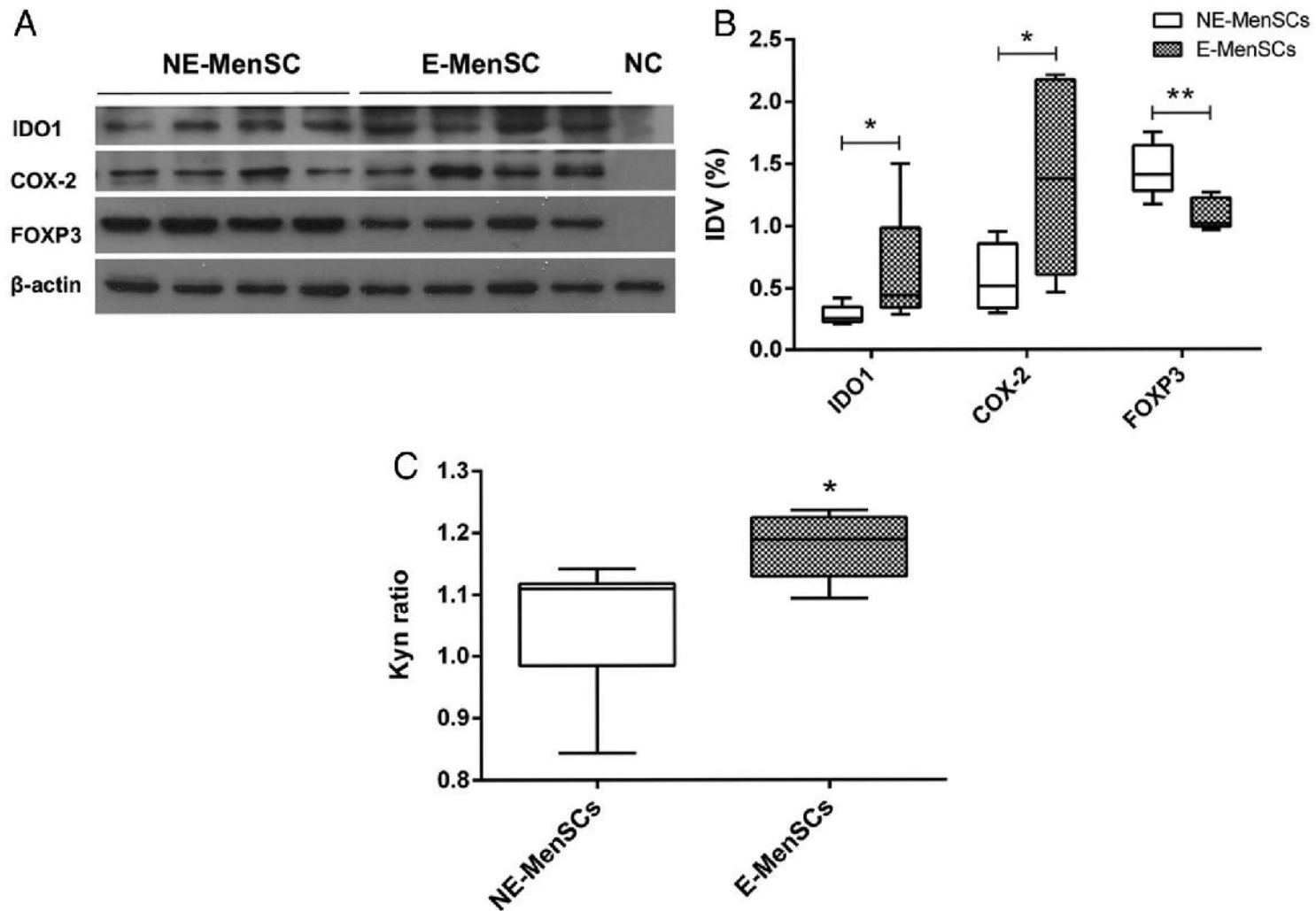
Cytokeratin



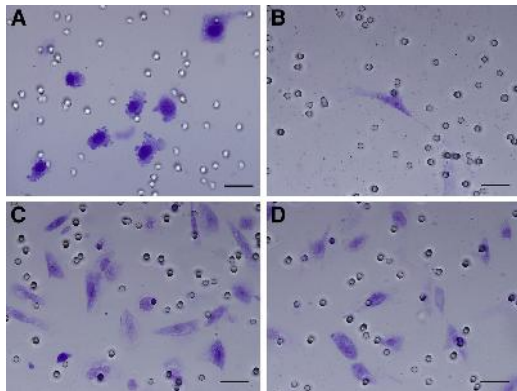
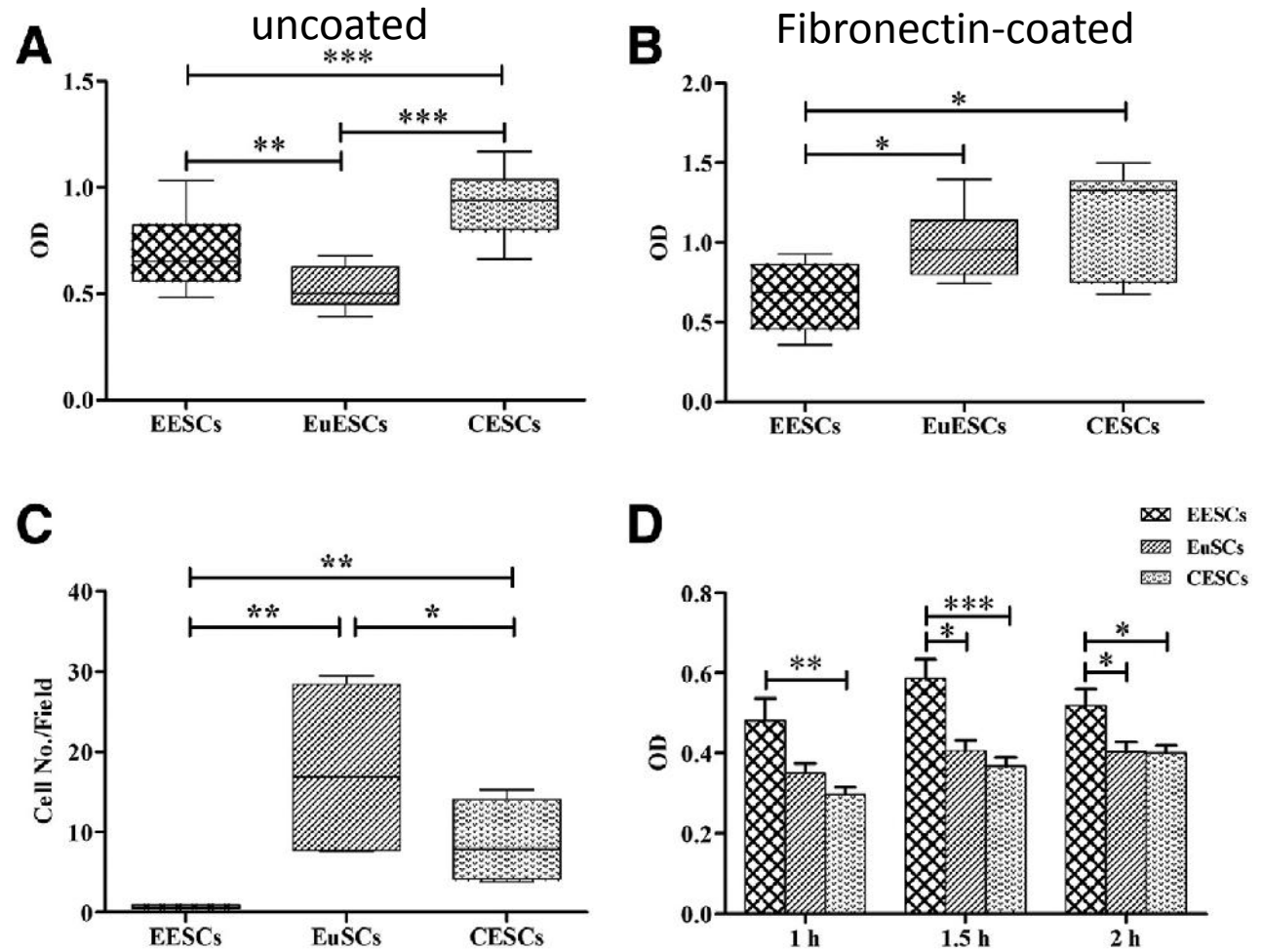
Morphological difference between NE-MenSCs and E-MenSCs.



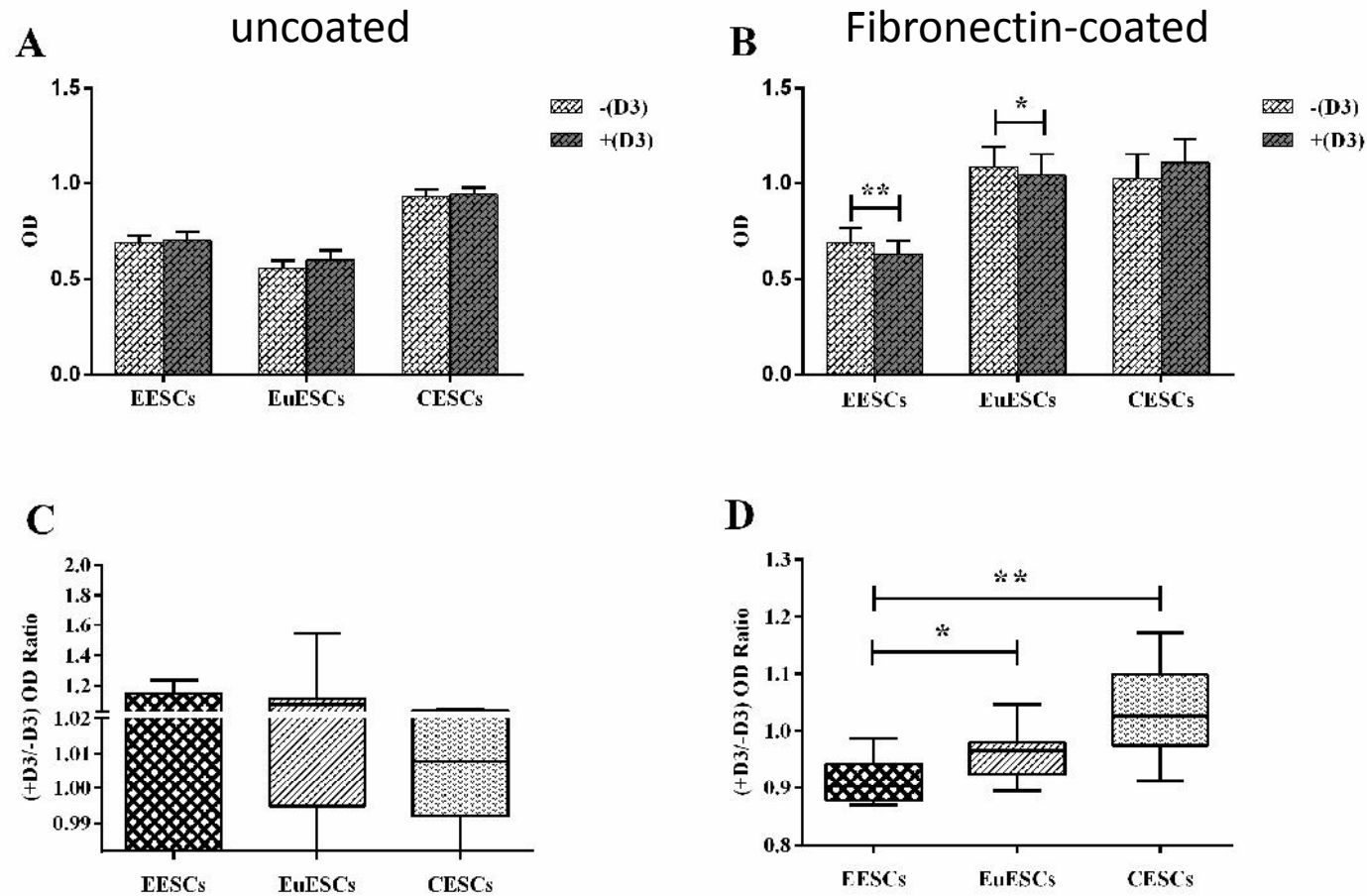
MenSCs of endometriosis patients have higher IDO1 and COX-2 and lower FOXP3 expression



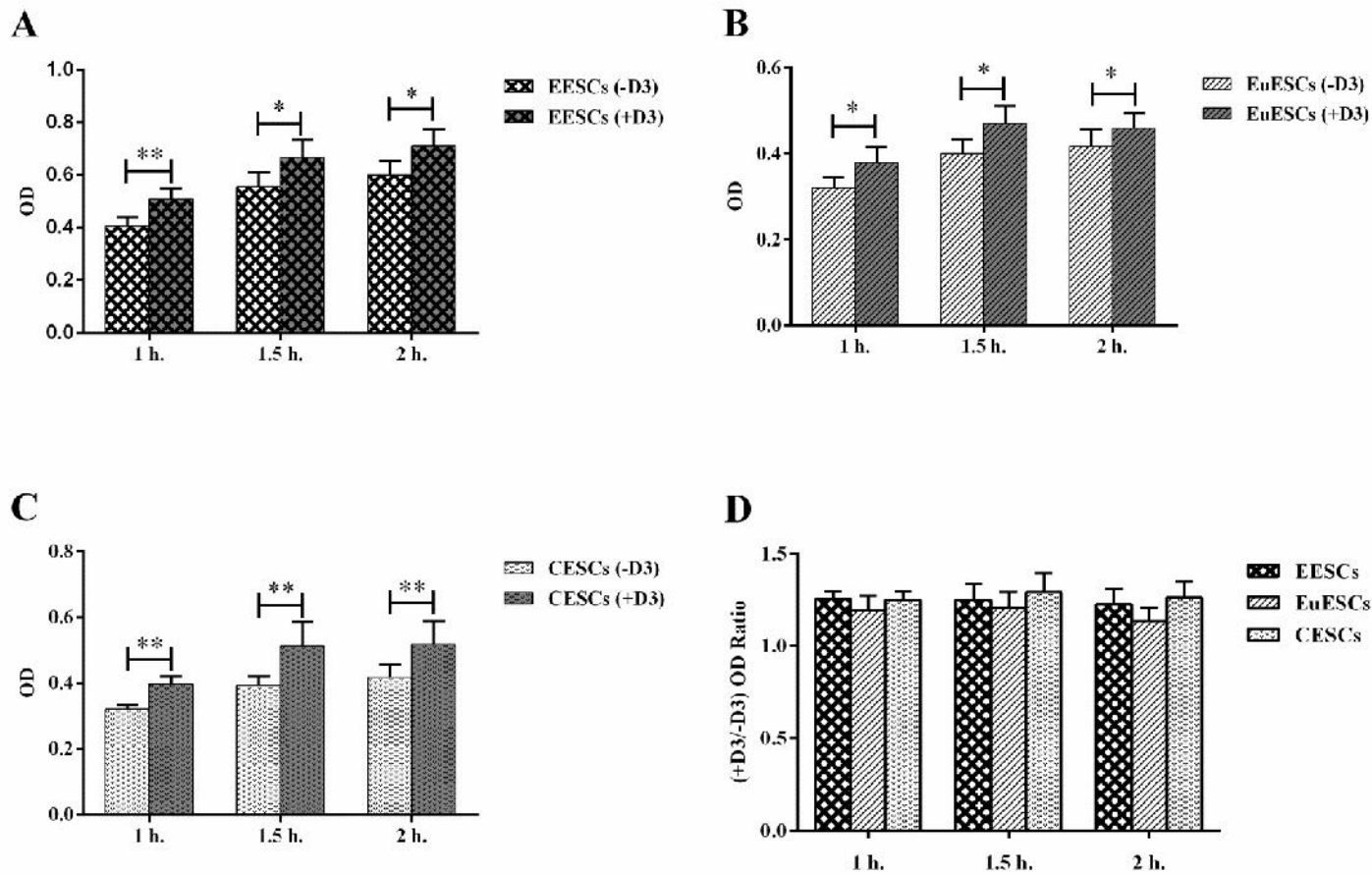
Comparative proliferation, invasion, and adhesion analysis of EESCs, EuESCs, and CESCcs



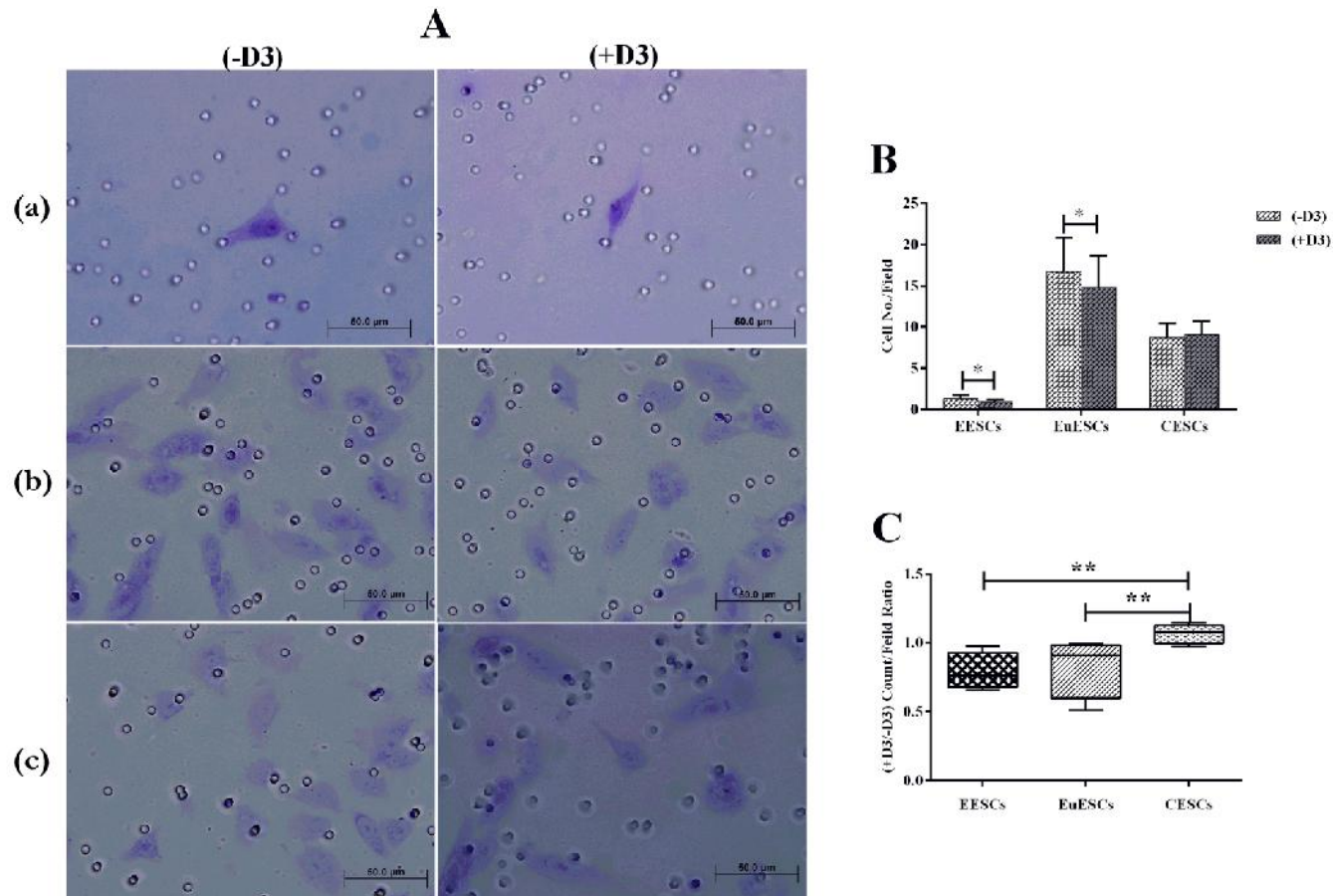
1,25(OH)₂D₃ selectively inhibits proliferation of EESCs and EuESCs



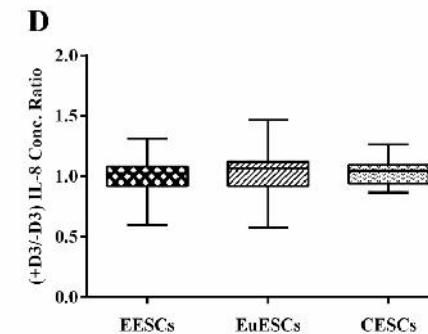
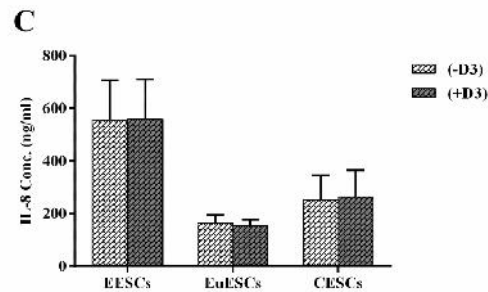
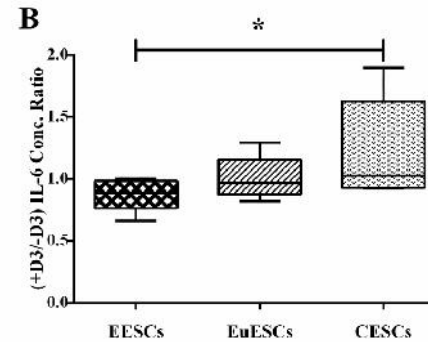
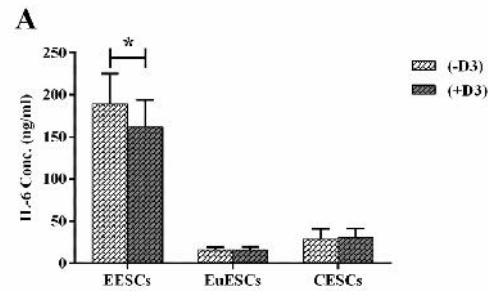
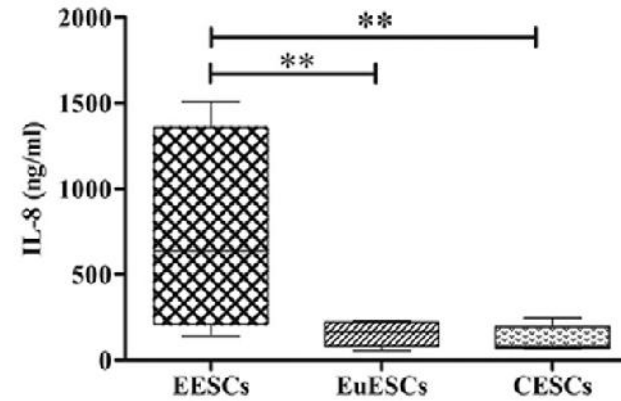
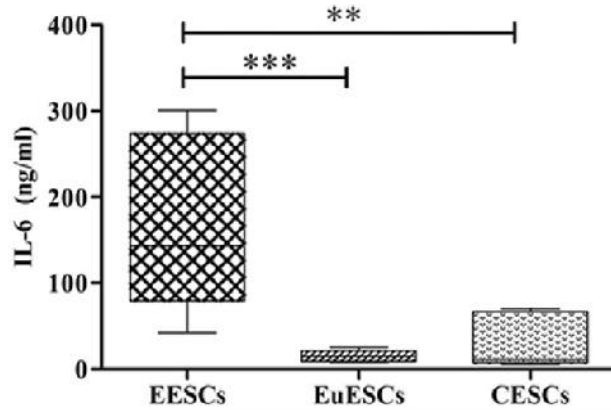
1,25(OH)₂D₃ increases adhesiveness of EESCs, EuESCs and CSECs



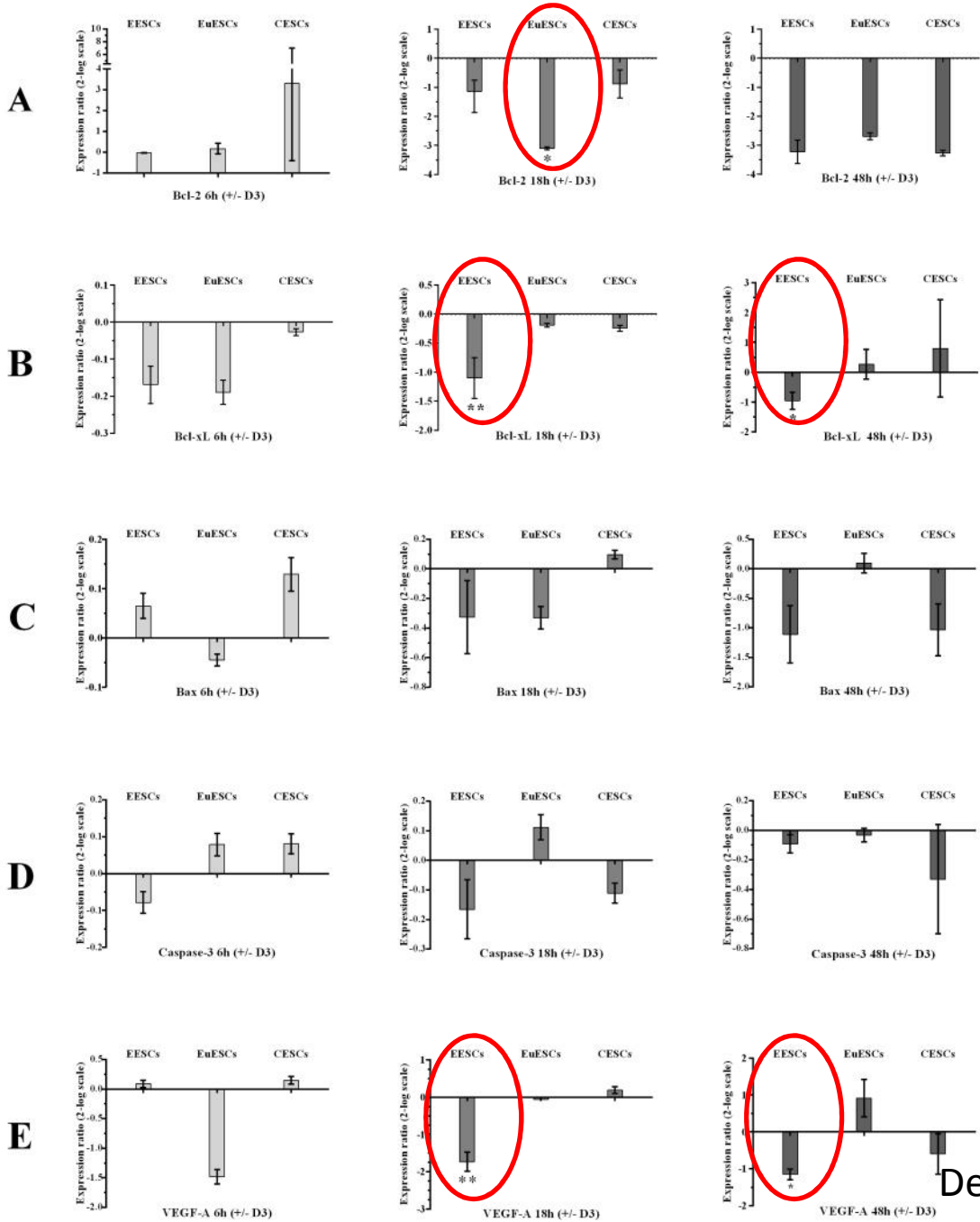
1,25(OH)₂D₃ differentially inhibits invasiveness of EESCs and EuESCs



1,25(OH)₂D₃ differentially inhibits IL-6 production by EESCs

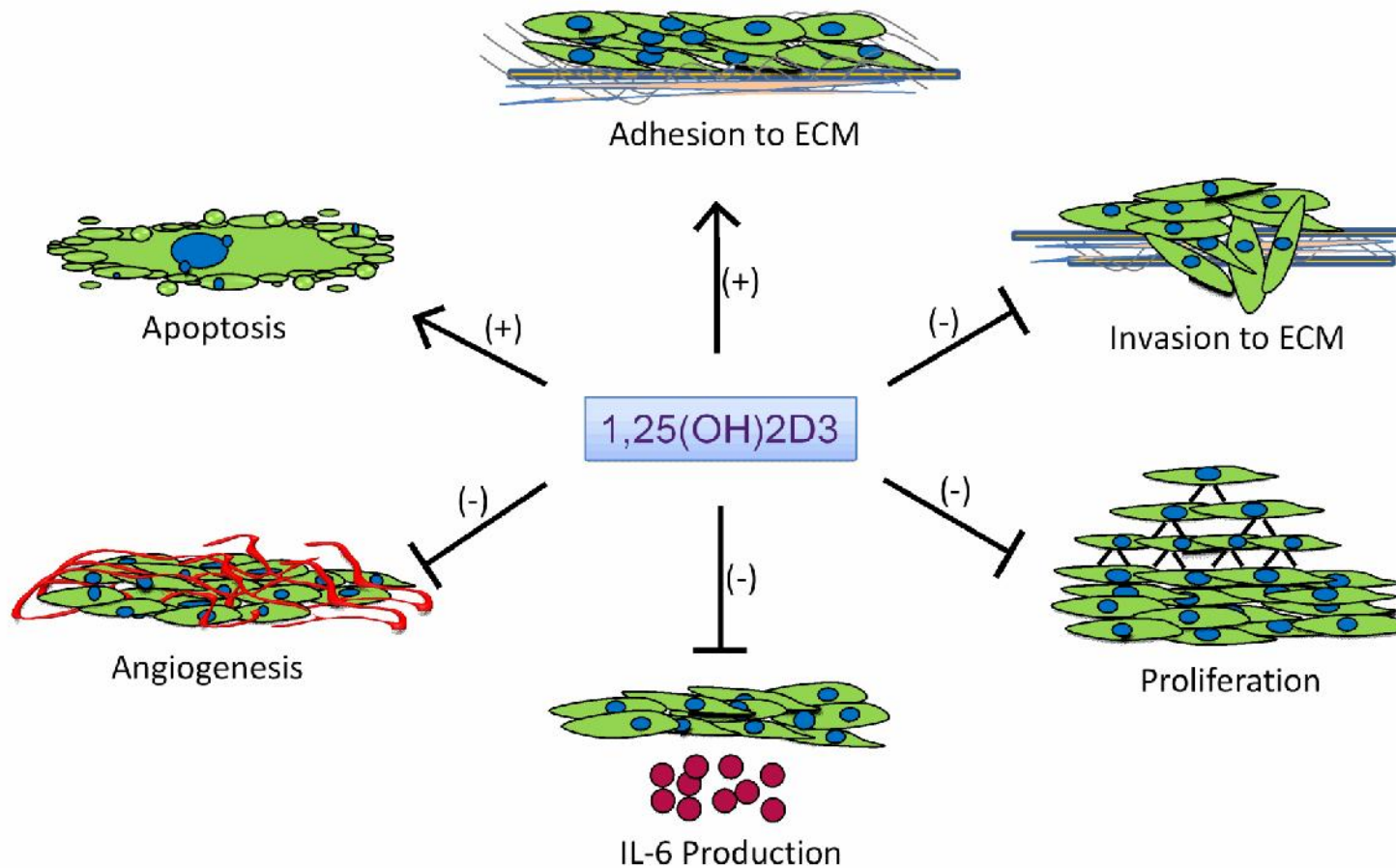


1,25(OH)₂D₃ upregulates apoptosis- related genes



Delbandi et al. Am J Reprod Immunol. 2016

Schematic representation of $1,25(\text{OH})_2\text{D}_3$ effects on endometriotic stromal cells



During 737,712 person-years of follow up over a 14-year period, it was found that **intakes of total and low-fat dairy foods** were associated with a **lower risk of endometriosis**.

Women consuming more than 3 servings of total dairy foods per day were 18% less likely to be diagnosed with endometriosis than those reporting 2 servings per day.

Predicted **plasma 25(OH)D level** was **inversely associated with endometriosis**.

Women in the highest quintile of predicted vitamin D level had a 24% lower risk of endometriosis than women in the lowest quintile.

Vitamin D, Polycystic Ovary Syndrome, and Insulin Resistance

PCOS is the **most common gynaecological endocrinopathy** in women of reproductive age, with a prevalence of 6–10% in the general population.

It is a multigenic disorder characterized by **increased ovarian and adrenal androgen secretion**; hyperandrogenic symptoms such as **hirsutism, acne, and/or alopecia**; **menstrual irregularity**; and **polycystic ovaries**.

Insulin resistance (IR) is common in PCOS women who are therefore at an increased risk of type 2 diabetes.

There is an increasing evidence that supports the contribution of **vitamin D deficiency to metabolic disturbances** in women with PCOS, including insulin resistance (IR), obesity, hypertension, and menstrual dysfunction.

Consistent evidence also suggests that **polymorphisms in the VDR gene** are associated with vitamin D deficiency in PCOS and its **metabolic and endocrine disturbances**.

The exact mechanisms underlying the association of vitamin D and IR are not fully understood.

Firstly, **vitamin D** may have a beneficial effect on insulin action by **stimulating the expression of insulin receptor** and thereby enhancing insulin responsiveness for glucose transport.

Secondly, vitamin D regulates extracellular and intracellular calcium that is essential for insulin-mediated intracellular processes in insulin responsive tissues

Finally, as vitamin D has a modulating effect on the immune system, hypovitaminosis D might induce a higher inflammatory response, which is again associated with IR.

Screening women with IR who are at risk of vitamin D deficiency and supplementation with vitamin D could be considered.

Vitamin D and Preeclampsia

Impaired placentation and maternal endothelial function are principal features of the pregnancy syndrome preeclampsia that affects 3–7% of all pregnancies.

Although the mechanisms through which low serum vitamin D levels can affect the risk of preeclampsia are still unclear, the causal relationship is biologically plausible.

Normal serum vitamin D levels help prevent hypertension through **suppression of the renin-angiotensin system**.

Vitamin D can influence blood pressure through the **suppression of vascular smooth muscle cell proliferation**.

It can **improve** endothelial cell-dependent **vasodilatation**.

Vitamin D may **modulate macrophage activity** and cytokine production.

The existence of gene transcript of **1 α -hydroxylase** and the finding of **VDR** expression in placental trophoblasts suggest a possible **autocrine loop of vitamin D** signaling within trophoblasts

It is believed that, during pregnancy, **1,25(OH) $_2$ D $_3$** may be produced not only by kidneys but also by **placenta trophoblasts**.

The active form of vitamin D, **1,25(OH) $_2$ D**, has been shown to **regulate the transcription** and function of genes associated with **placental invasion, normal implantation, and angiogenesis**.

A recent **meta-analysis** and several observational studies show a **significant relationship between vitamin D deficiency and an increased risk for preeclampsia**.

Supplementing pregnant women with **vitamin D** in a single or continued dose increases serum 25-hydroxyvitamin D at term and may **reduce the risk of pre-eclampsia, low birthweight and preterm birth.**

However, when **vitamin D and calcium** are combined, the **risk of preterm birth is increased.**

Conclusion

ویتامین D خوب است.

من ویتامین D را دوست دارم.

With special thanks to:

M. Tavakoli, Ph.D.

M. Shahbazi, Ph.D.

A.A. Delbandi, Ph.D.

Sh. Nikoo, Ph.D.



Vitamin D and Gestational Diabetes Mellitus

GDM is a condition of abnormal maternal glucose tolerance that occurs, or is detected for the first time, during pregnancy. Pregnancy is a status in which the mother undergoes physiological insulin resistance, which helps the fetus absorb more nutrients.

Maternal postprandial hyperglycaemia is the reason why the fetus can take in more carbohydrates and amino acids via the placenta, thanks to a carrier passage, the functioning of which is facilitated by the different gradient (typically facilitated transport). If the mother is unable to compensate with an increase of pancreatic β -cell insulin secretion, GDM is derived from this metabolic condition.

Women affected by GDM generally demonstrate in the puerperium, and/or later in life, a maintenance of high levels of insulin resistance, which is the effect of β -cell dysfunction, and suggests that GDM is a transient manifestation of longstanding metabolic impairment with a predisposition to reappear in the future.

There is a strict connection between glucose metabolism and vitamin D pathways: it is widely accepted, for example, that this vitamin and PTH play a key role in the extracellular homeostasis of calcium, and moreover that patients affected by hyperparathyroidism develop more frequently diabetes mellitus type 2 with respect to the general population.

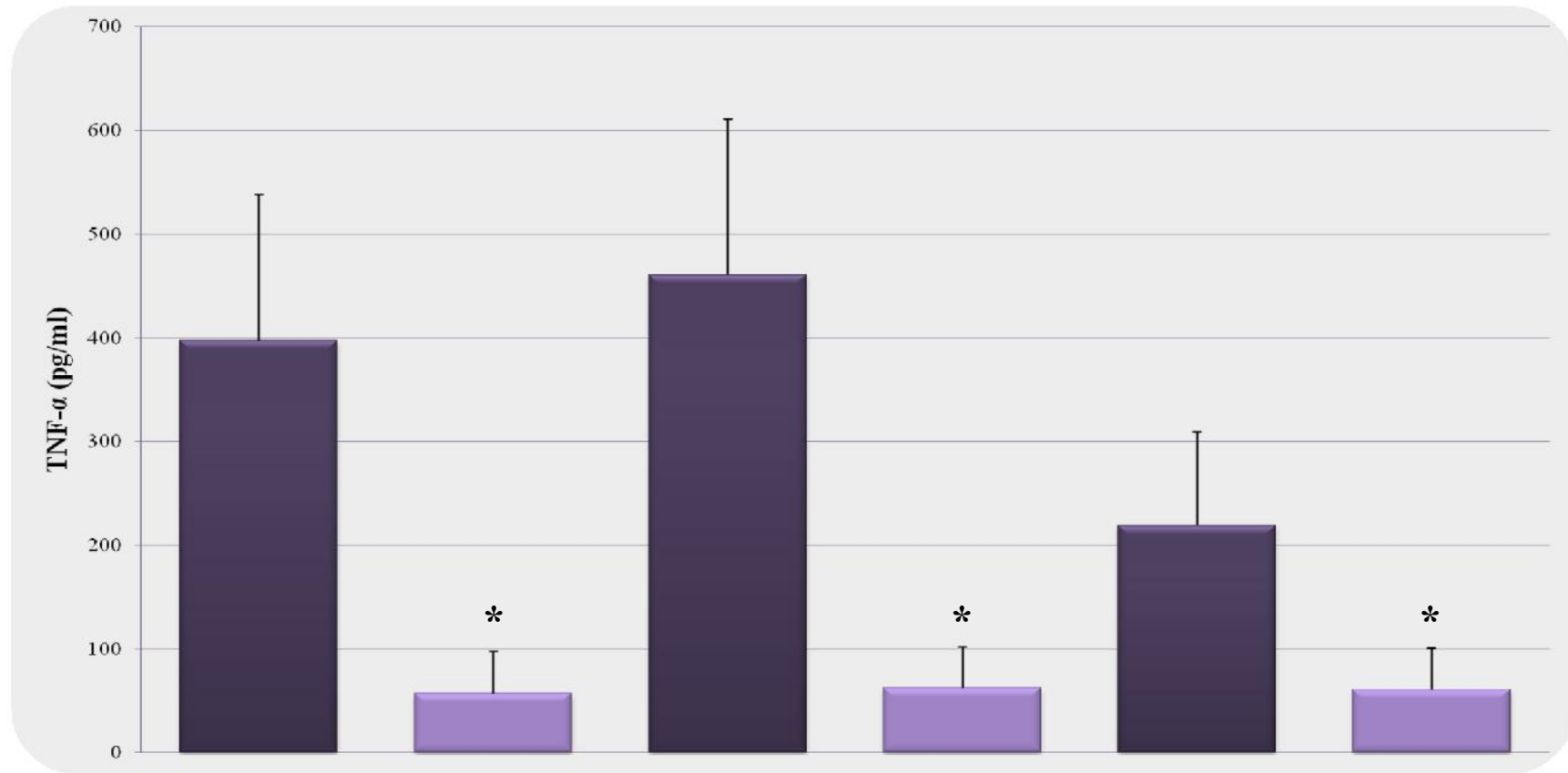
Since $1,25(\text{OH})_2\text{D}$ is able to induce insulin secretion and to decrease insulin resistance, low levels of this vitamin are associated with the developing of GDM.

Prevalence of severe vitamin D deficiency (<12.5nmol/L; <5 ng/mL) in GDM patients was higher than in normoglycaemic pregnancies and found a strong correlation between the HOMA index and serum levels of vitamin D.

Moreover, each 12,5 nmol/L (5 ng/mL) decrease in 25(OH)D concentrations was related to a 1.29-fold increase in GDM risk.

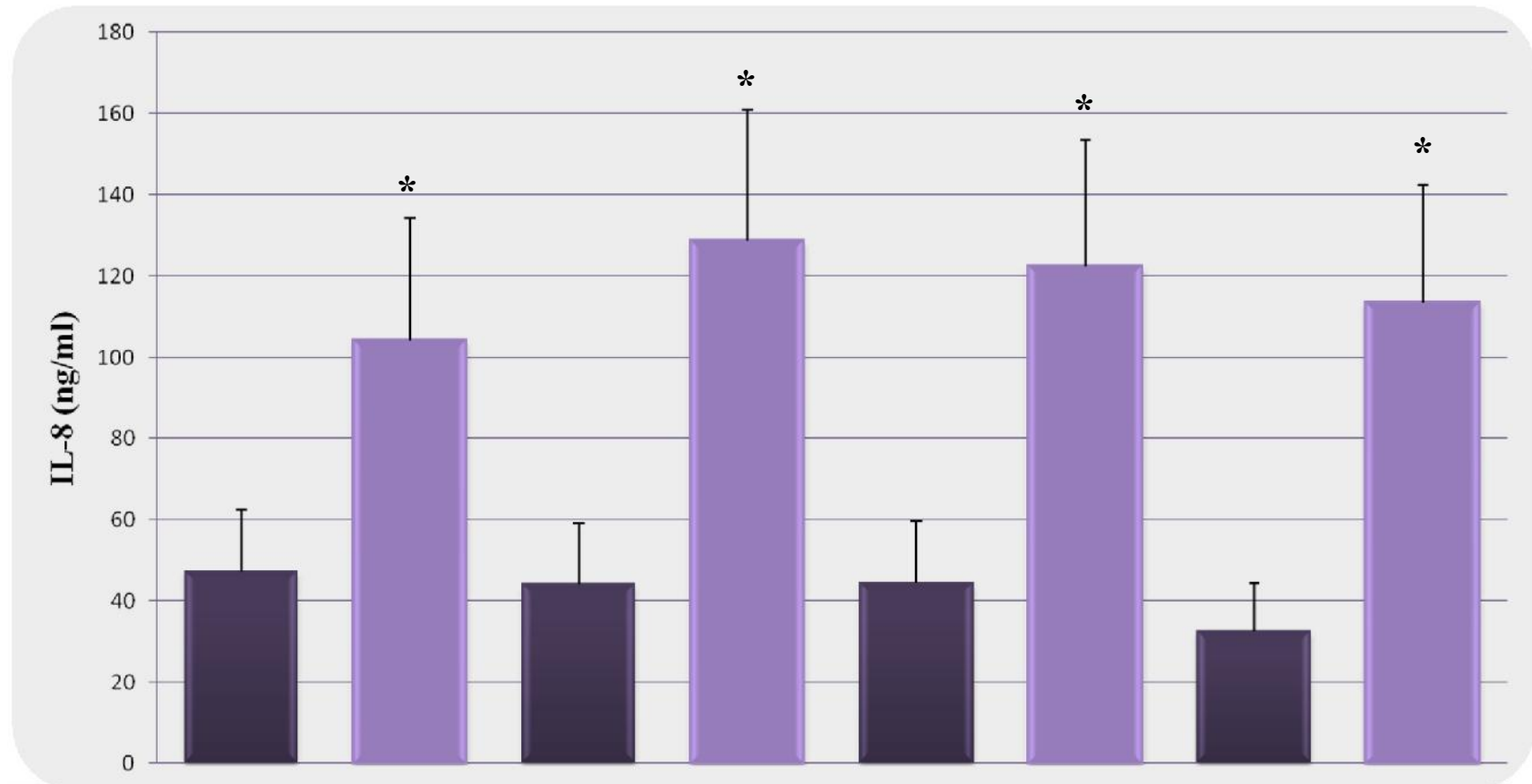
Taken together, all these results allow us to underline the strict correlation between vitamin D and glucose metabolisms, even if further studies based on larger population are needed to get crystal clear evidence about the topic.

Effect of $1,25(\text{OH})_2\text{D}_3$ on TNF- α production by ESCs stimulated with different concentrations of LPS



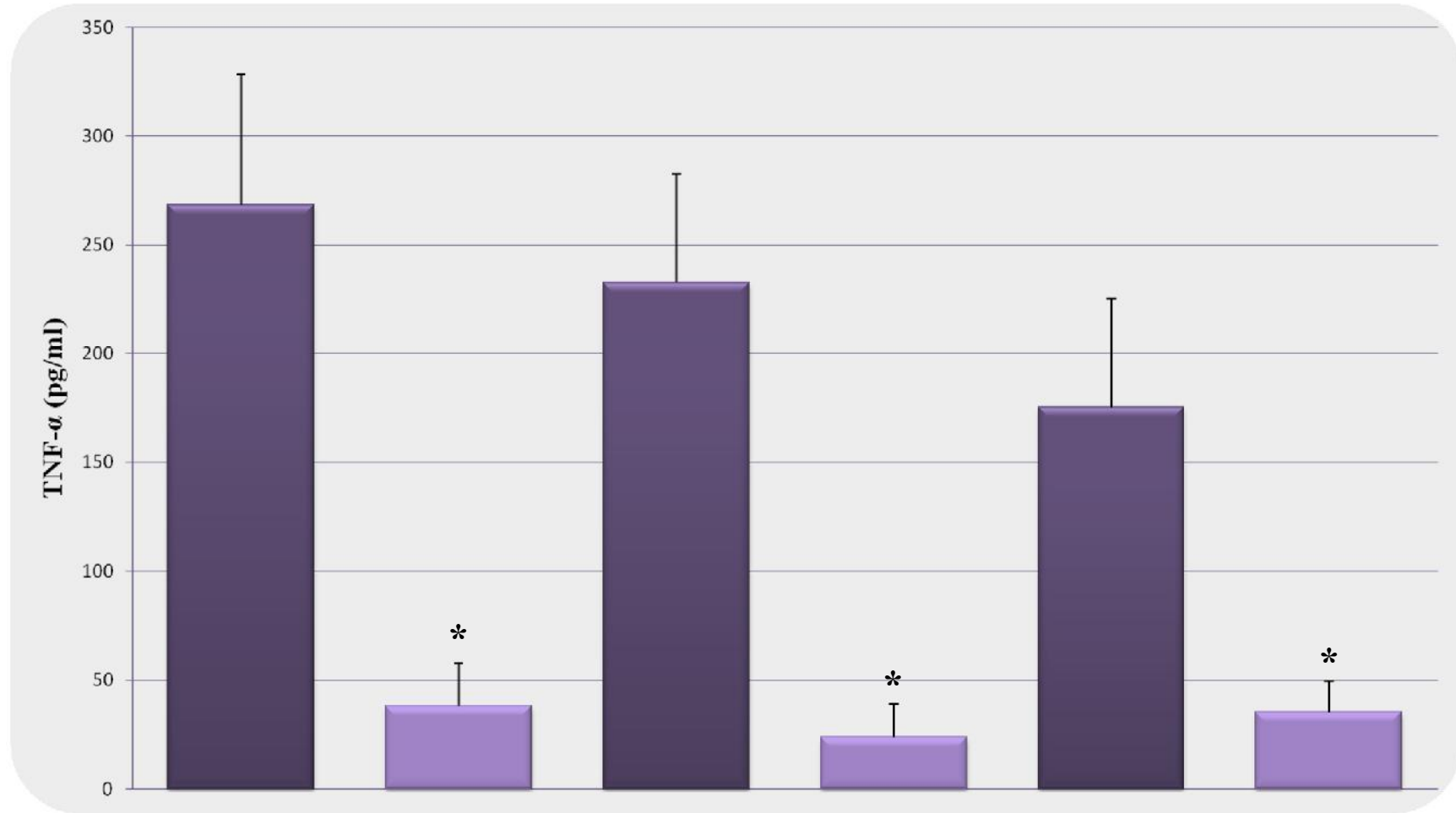
LPS (1000 ng/ml)	+	+	-	-	-	-
LPS (100 ng/ml)	-	-	+	+	-	-
Vit D3	-	+	-	+	-	+

Effect of 1,25(OH)₂D₃ on IL-8 production by ESCs stimulated with different concentrations of LPS



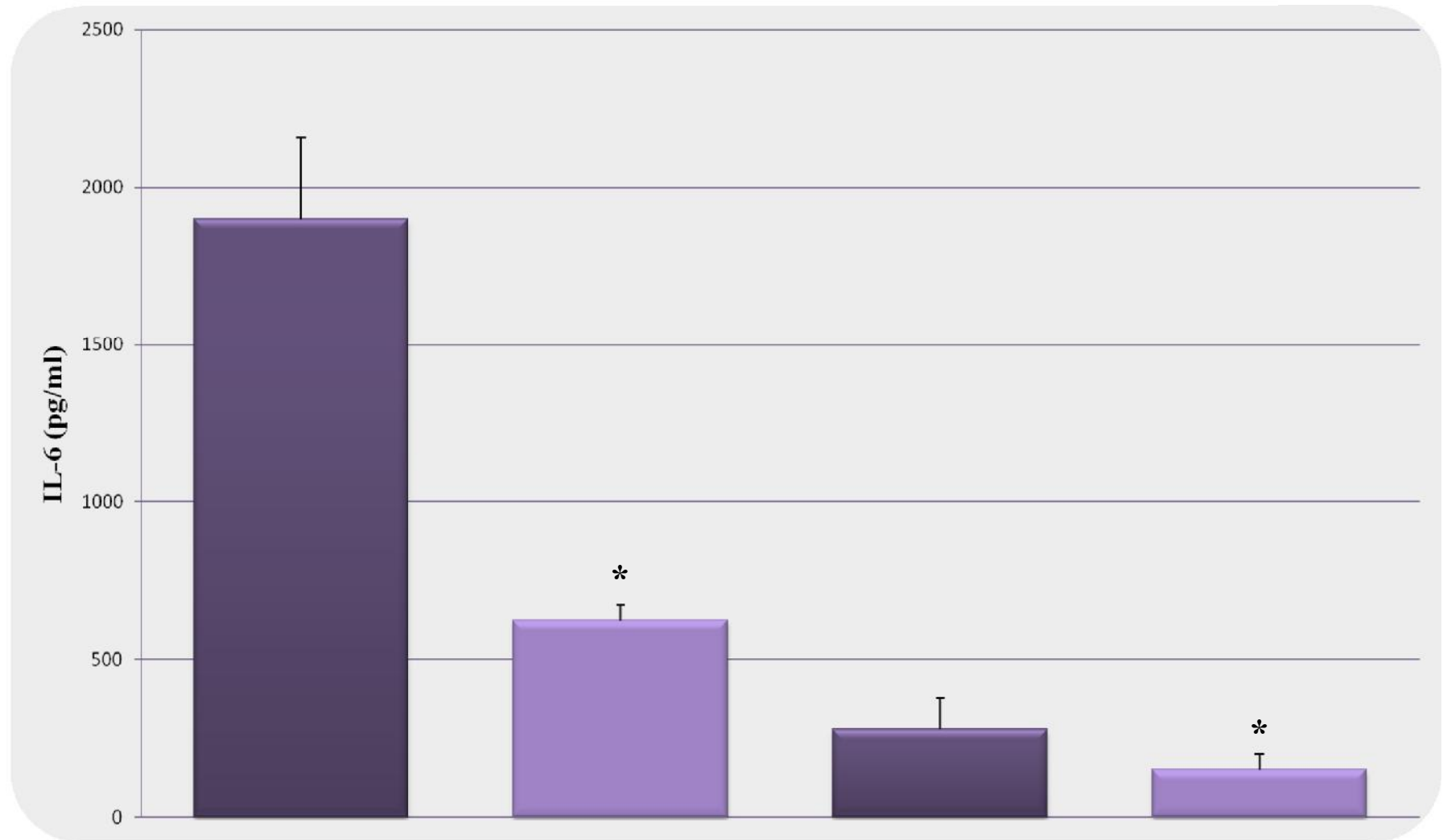
LPS (10000 ng/ml)	+	+	-	-	-	-	-	-
LPS (1000 ng/ml)	-	-	+	+	-	-	-	-
LPS (100 ng/ml)	-	-	-	-	+	+	-	-
Vit D3	-	+	-	+	-	+	-	+

Effect of 1,25(OH)₂D₃ on TNF production by WECs stimulated with different concentrations of LTA



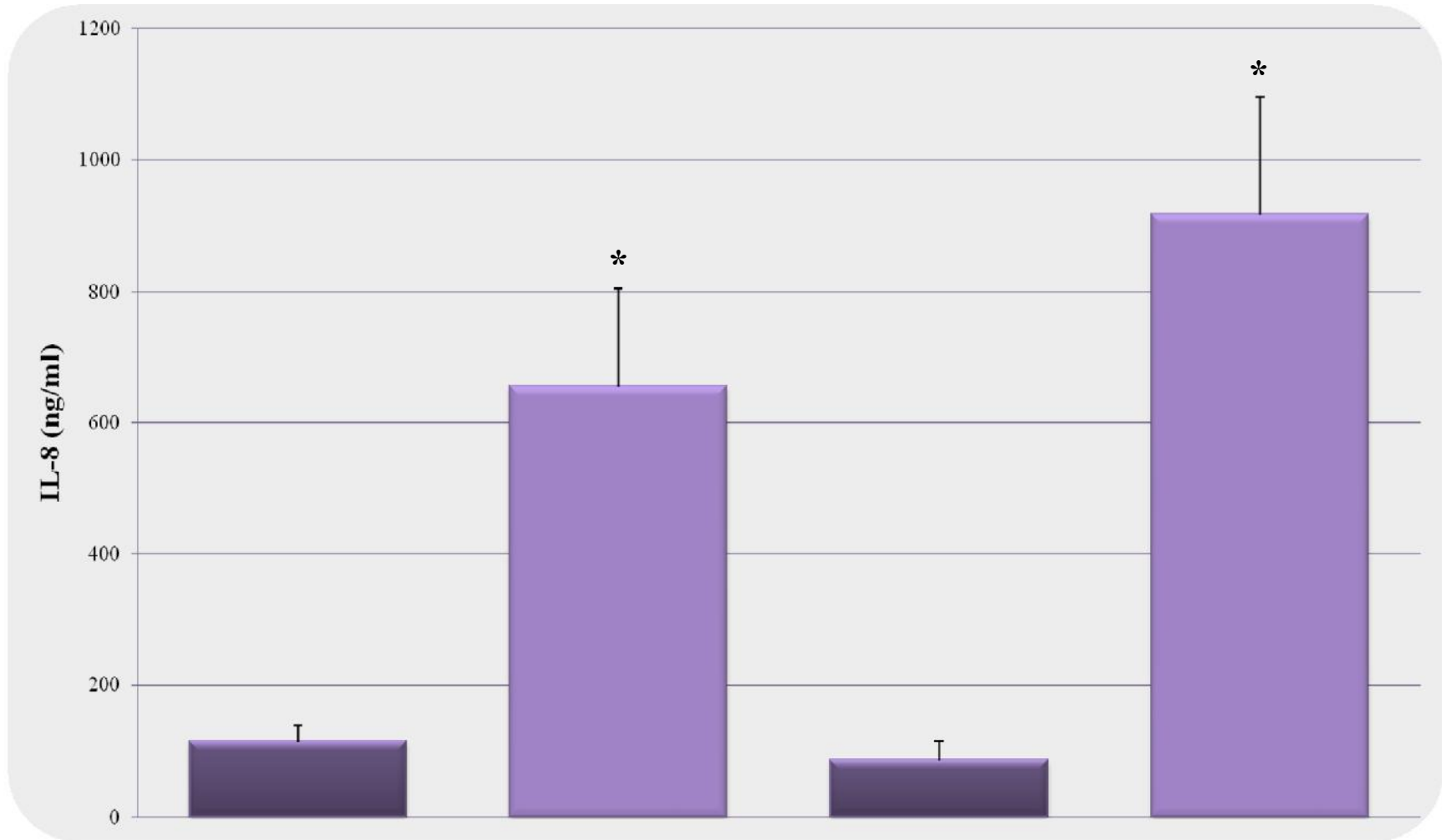
LTA (10000 ng/ml)	+	+	-	-	-	-
LTA (1000 ng/ml)	-	-	+	+	-	-
Vit D3	-	+	-	+	-	+

Effect of 1,25(OH)₂D₃ on IL-6 production in LTA- stimulated WECs



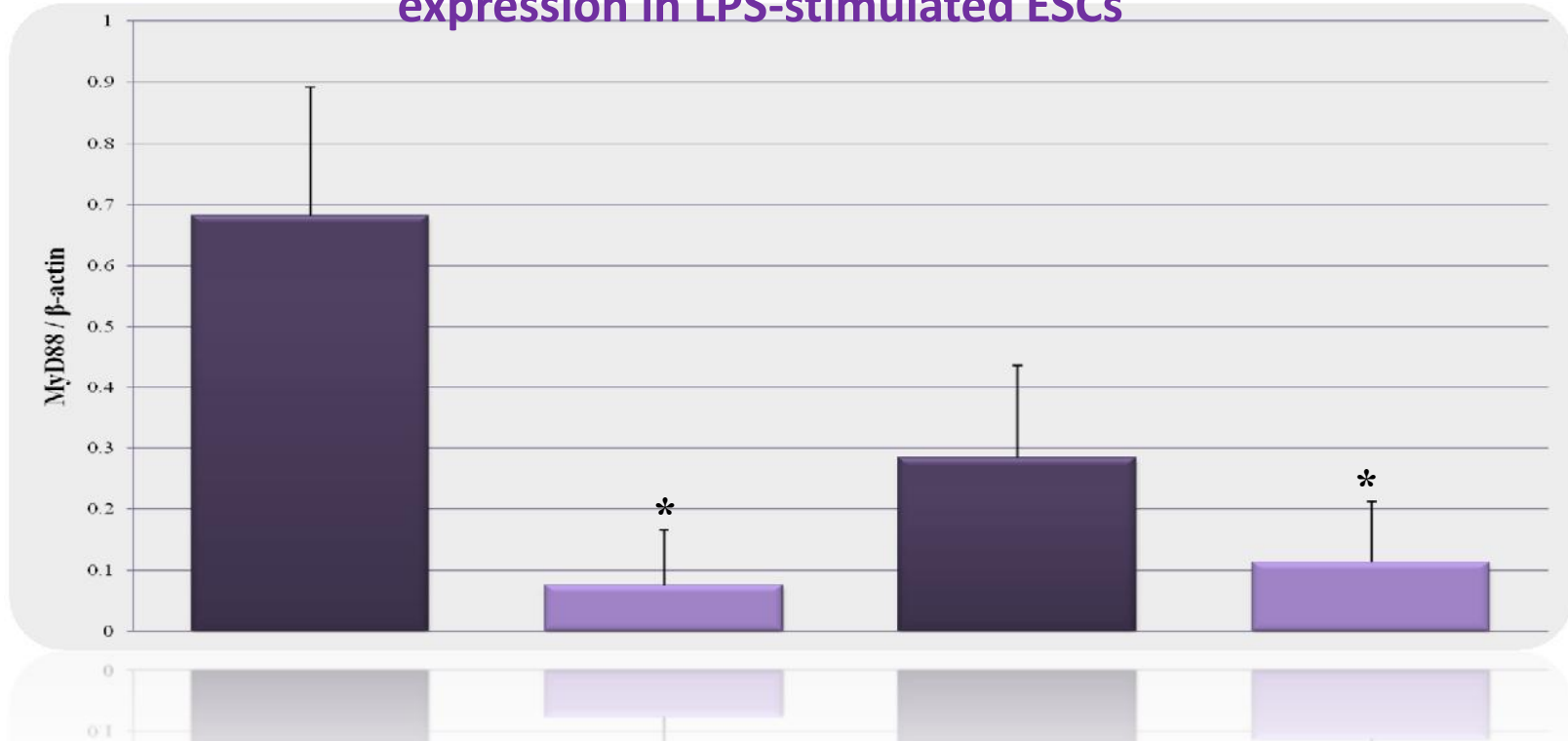
LTA (1000 ng/ml)	+	+	-	-
Vit D3	-	+	-	+

Effect of 1,25(OH)₂D₃ on IL-8 production in LTA-stimulated WECs

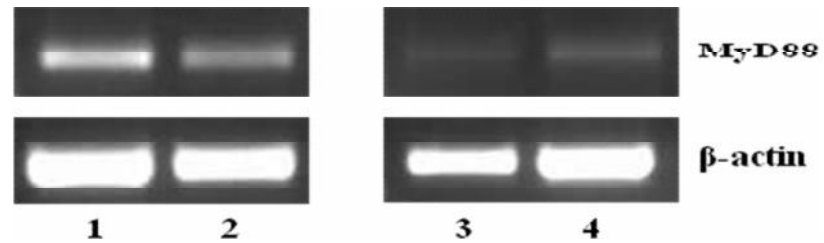


LTA (1000 ng/ml)	+	+	-	-
Vit D3	-	+	-	+

Effect of 1,25(OH)₂D₃ on MyD88 gene expression in LPS-stimulated ESCs



LPS (100 ng/ml)	+	+	-	-
Vit D3	-	+	-	+



Taken together, we strongly believe that vitamin D₃ supplementation could potentially reduce the burden of infection- or immunologic-induced RSA and its level, therefore, should be checked and if insufficient be prescribed for women with RSA.