

The role of vitamin D in impaired fertility treatment

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Abstract

Vitamin D is currently in the scope of research in many fields of medicine. Despite that its influence on health remains uncertain. This paper presents the review of the publications concerning the role of calciferol in reproduction processes and its significance in infertility therapy covering topics of polycystic ovary syndrome, endometriosis infertility, myoma infertility, male infertility, premature ovary failure and in vitro fertilization techniques. The results of latest research articles in those fields has been discussed and summarized.

The deficiency of vitamin defined as the concentration of 25-hydroxycalciferol <20 ng/ml is frequently noted in patients of fertility clinics. Serum vitamin D concentration in healthy women is higher comparing to PCOS patients. The supplementation with vitamin D should be applied in the schemes of PCOS treatment both due to an improved insulin resistance and the

results of infertility treatment. The explanation of vitamin D activity mechanism in patients with PCOS requires further research. Vitamin D have direct effect on AMH production, and thus increase longer maintenance of ovarian reserve in the patients with its higher concentration. The occurrence of uterine myomas in the group with vitamin D deficiency was evaluated as much higher comparing to controls. On the other hand it is supposed that high concentration of calciferol may be related to an impaired elimination of endometrial cells passing to peritoneal cavity via ovarian reflux causing endometriosis. In male infertility both low (<20 ng/ml) and high (>50 ng/ml) concentration of vitamin D in serum negatively affects spermatozoa number per ml of semen, their progressive movement and morphology.

Significant differences as a response on ovulation stimulation, number and quality of embryos depending on vitamin D concentration were not observed in none of the analyzed papers concerning the role of vitamin D in in vitro fertilization (IVF). Better results in patients without calciferol insufficiency are explained by reports about high concentration of vitamin D and its metabolites in human in decidua collected in the 1st trimester of pregnancy which suggests its contribution in proper implantation and local immunological preference of the embryo. It is accepted that the treatment requires vitamin D concentration below 20 ng/ml (up to 50 nmol/l), especially in obese women, these with insulin resistance and small ovarian reserve and in men with oligo- and asthenozoospermia.

Introduction

Vitamin D (calciferol) has been placed in the range of researchers interest just since the year 1920, after Mellanby noted the relationship between cod liver consumption and rachitis symptoms regression (Mellanby, 1919). Nowadays this diseases is almost not observed in Europe, however in the 1960' it was the frequent reason of disability and numerous obstetric complications (Troszyński, 2009). Many papers concerning the role of vitamin D in organism

homeostasis have been published last years, and they fall outside previous scope of its contribution in osseous turnover. The database of the US National Library of Medicine National Institutes of Health (PubMed) contains 60377 articles concerning calciferol. Based on the results of the study concerning the role of vitamin D in immunology and cancers development, there is a search for evidences of profitable supplementation effect in the therapy of numerous diseases differing with etiopathogenesis such as psoriasis, diabetes, leukemias or osteoporosis (Seremak-Mrozikiewicz et al, 2009). The research on vitamin D role in reproduction processes modulation seems to be especially interesting. The conclusions from these studies are presumably of a significant practical meaning, especially in infertility therapy.

The term vitamin D is used both in respect to vitamin D₂ (ergocalciferol) and D₃ (cholecalciferol). Both are synthesized in the skin from 7-dehydrocholesterol under an influence of an activity of ultraviolet B radiation. The source of vitamin D of a minor significance is also food and diet supplements. Vitamin D₃ is contained in sea fish fat and cod liver oil, while D₂ in plants and mushrooms. In blood, vitamin D is transported by vitamin D-binding protein (VDBP). In the liver vitamin D is metabolized by hydroxylases included in cytochrome 450 to the form of active 25-hydroxyvitamin D (25[OH]D) (Prosser, Jones, 2004). Determination of 25[OH]D concentration in blood serum is mainly used for an assessment of vitamin D deficiencies. There are however some discussions concerning an optimum concentration of vitamin D in blood serum, but it is commonly accepted it should be ≥ 20 ng/ml (≤ 50 nmol/l) (Rosen et al., 2012). 25[OH]D is metabolized in kidneys by 1 α -hydroxylase (CYP27B1) to an active form of 1,25-dihydroxyvitamin D. Chemical structure of vitamin D is similar to the structure of steroid hormones, and like them it acts via nuclear receptor (VDR, vitamin D receptor) (Christakos et al., 2007). Due to above reasons it is included to hormones. Luk et al. (1979), based on the study conducted in vitro, demonstrated

that both decidua and placenta produce active metabolites of vitamin D, 1,25[OH]₂D and 24,25-dihydroxyvitamin D (24,25[OH]₂D). The presence of 1 α -hydroxylase and VDR receptors in these tissues, and also in the cells of ovary, endometrium, pituitary gland was noted slightly later, and its effect on the functions of ovary granulosa cells and the role in steroidogenesis as well as reproduction and immune system regulation were described (Perez-Fernandez et al., 1997; Evans et al., 2006; Parikh et al., 2010; Torrealday et al., 2012). The presence of VDR in males was demonstrated in testicles and spermatozoa (Habib et al. 1990; Aquila et al., 2009). An activity of 1 α -hydroxylase in spermatozoa cells points a local synthesis of vitamin D and its autocrine and paracrine activity (Fig. 1). 1,25[OH]₂D increases an intracellular concentration of Ca⁺² ions, and affects an activity of acrosine, one of the enzymes involved in acrosome reaction (Aquila et al., 2009). However, despite a high number of fundamental research, the available data concerning biological effect of vitamin D on fertility are very poor and often unequivocal.

Methods

This paper presents the review of the publications concerning the role of calciferol in reproduction processes and its significance in infertility therapy. Under the key words “vitamin D, infertility”, the PubMed database contains 85 articles (23.09.2013), among which 25 publications directly concerning the issues are discussed.

Result

The results of the studies concerning vitamin D role in fertility impairments and its significance for the effects of therapy in women with polycystic ovary syndrome (PCOS), uterine fibroids, infertility, in men with improper semen parameters and in case of in vitro treatment failure were discussed and summarized.

Discussion

Polycystic ovary syndrome

PCOS is the most often diagnosed endocrinological disorder in women in reproductive period. It is recognized based on Rotterdam criteria or according to Androgen Excess Society criteria (Azziz, Carmina, et al., 2006; Azziz, 2006). PCOS is not a uniform syndrome, is multi-symptomatic, and it is often difficult to determine the cause-effect relations in its pathogenesis between clinical symptoms and biochemical disorders (Thomson et al., 2012).

The results of a few papers point the significance of vitamin D in PCOS pathogenesis. The deficiency of vitamin defined as the concentration of 25-hydroxycalciferol <20 ng/ml was noted in 65-87% of the patients with PCOS (Patra et al., 2012). This may be explained by more frequent occurrence of obesity, possibility of vitamin D accumulation in adipose tissue, and sunbathing avoidance, especially in case of the women with hirsutism. However, no relationship with all symptoms of this syndrome was demonstrated. Negative correlation was observed with insulin resistance, metabolic syndrome symptoms, the value of systolic and diastolic blood pressure and lipids metabolism disorders (Wehr et al., 2009; Yildizhan R , et al., 2009; Patra et al., 2012; Ott et al., 2012). As may be concluded from the study, obesity is related both to insulin resistance and vitamin D deficiency, but a deficiency of vitamin D itself may decrease cells susceptibility on insulin (Fig. 2). Vitamin D affects glucose transport to the cell via stimulation of an expression of insulin receptor and insulin secretion (Christakos, 2013).

One of the main problems of patients with recognized PCOS is infertility. In the presented literature review, attention should be paid to the study analyzing an influence of vitamin D deficiency on the results of ovulation stimulation using clomifene citrate (CC). Ott et. al. examined basic parameters of calcium balance in an organism of 91 patients treated due to PCOS. They observed an ovulation in 57.1% of the patients, and pregnancy in 26.9% (24/91)

after stimulation with 50 mg clomifene. Analysis of the concentrations of parathormone, androstenedione, luteinizing hormone (LH), follicle stimulating hormone (FSH), sex hormone binding protein (SHBG), calcium in serum, vitamin D3 and body weight unequivocally demonstrated positive correlation between proper development of ovarian follicles, percentage of pregnancies as well as proper body mass index (BMI) and calciferol concentration (Ott et al., 2012).

However, is vitamin D management especially significant in patients with PCOS? Only pilot study has been conducted so far, which compared an effect of pharmacological inhibition of insulin secretion on vitamin D concentration in healthy women and these with diagnosed PCOS. Both groups did not differ in terms of age and BMI. The study demonstrated considerably lower concentration of vitamin D in patients with PCOS, which may point its significance in PCOS pathogenesis (Nestler et al., 2012). It seems in the light of reports presented that the supplementation with vitamin D should be applied in the schemes of PCOS treatment both due to an improved insulin resistance and the results of infertility treatment. The explanation of vitamin D activity mechanism in patients with PCOS requires further research.

The reports from last in vitro and in vivo studies suggest an influence of vitamin D deficiency on a development of uterine fibroids and endometriosis. The presence of two above pathologies is often observed in women with infertility (Paffoni et al., 2013; Di Rosa et al., 2012).

Uterine myomas

Paffoni et al. conducted the study including 128 patients treated due to infertility, in which at least one uterine fibroid of a diameter above 10 mm was detected, and 256 patients in the control group, at a similar age (± 1 year), and demonstrated lower concentrations of vitamin D

in women with fibroids (18.0 ± 7.7 vs 20.8 ± 11.1 ng/ml, respectively, $p = 0.010$). The risk (OR) of fibroids occurrence in the group with vitamin D deficiency was evaluated as 2.4 (95%; RR 1.2-4.9), ($p=0.16$) (Paffoni et al. 2013). These data does not demonstrate however any statistical significance but clear tendency. There is a need of further cohort and biochemical research explaining the correlation between vitamin D concentration and uterine fibroids development.

Endometriosis

The papers concerning endometriosis occurrence in the patients with high calciferol concentration seem to be very interesting. This is related to an evidenced immunomodulating effect of vitamin D. It is supposed that its high concentration may be related to an impaired elimination of endometrium cells passing to peritoneal cavity via ovarian reflux (Di Rosa et al., 2012). In the study published in Human Reproduction, Somigliana et al. examined the concentration of $25(\text{OH})\text{D}_3$, $1,25(\text{OH})_2\text{D}_3$, Ca^{+2} in 87 patients with endometriosis and 53 healthy women. An analysis demonstrated significantly higher concentration of vitamin D in the examined group (24.9 ± 14.8 ng/ml vs. 20.4 ± 11.8 ng/ml, $p=0,05$), and considerably higher chance of diseases occurrence among the patients with vitamin D concentration in serum exceeding the level of 28.2ng/ml (75 percentile), with OR=4.8 (1.7-13.5). No differences in the concentration of vitamin D and Ca^{+2} were noted depending on menstrual cycle phase. The differences in concentration of calcium and active vitamin precursor, $1,25(\text{OH})_2\text{D}$ did not reach a statistical significance, however the trend pointing their positive correlation with the disease occurrence was distinct (Somigliana et al., 2007). This publication confirms the opinions that an increased cholecalciferol reserve increases the risk of endometriosis occurrence.

Premature ovarian failure

Next group of the patients involved in the study on an influence of vitamin D on fertility includes the women affected with the premature ovarian failure, starting the menopausal period before 40th year of life. Anti-Müllerian hormone (AMH) was accepted as the biochemical marker allowing to confirm the diagnosis of this syndrome (Visser et al., 2006). Its physiological level after temporary fluctuations in childhood period is stabilized at the age of about 8 years, and then decreases gradually from about 25th year of life to menopause occurrence (Kesley et al., 2011). AMH is produced by ovary granulosa cells irrespective of stimulation with gonadotropins, and its role in reproduction mainly involves stimulation of primary follicles in the ovary and making them susceptible on stimulation with FSH (Seifer, Maclaughlin, 2007), and for this reason it is often considered as the parameter determining ovarian reserve and prognostic marker of the results of ovulation stimulation in assisted reproduction procedures. Direct effect of vitamin D on AMH level was confirmed in recently conducted in vitro study (Malloy, 2009). Mehri et al. (2012) examined the correlation between the concentration of 25-hydroxycalciferol and AMH. This study was a part of larger, multi-center project dealing with a health of HIV positive patients, conducted in the United States. The effect of infection with the virus both on vitamin D and AMH concentration was excluded in this study. In turn, an unequivocal correlation between these two compounds concentrations was demonstrated in the groups of patients above 40th year of life. The authors of the study suggest a direct effect of vitamin D on AMH production, and thus longer maintenance of ovarian reserve in the patients with its higher concentration.

Male infertility factor

Also andrologists joined the worldwide research on vitamin D role in human reproduction. The review of the literature available provides a very interesting, but not always unequivocal conclusions in this range. The probability of a negative effect of vitamin D on fertility was suggested after a discovery of a molecular similarity of its transporting protein VDBP

(Vitamin D Binding Protein) to antisperm antibodies, however the experiments conducted demonstrated lack of reaction of specific IgG with VDBP (Yu HM, 1994). So far it was the only publication concerning this issue and its significance is difficult to explain. Further research in this range with an application of modern laboratory methods seem to be inevitable.

The concentration of bioavailable testosterone, vitamin D, and bone mineral density (BMD) was compared in the study of Yang et al. in the group 355 men with male infertility (patients with oligo-, astheno-, terato- and normospermia) and the group of 155 healthy men at the same age. Considerably lower serum testosterone concentration was noted in the examined group, as well as lower bone density measured densitometrically in the lumbar spine and ilium bone ($p < 0.05$). The concentration of bioavailable testosterone and 25-hydroxycalciferol constituted an independent factor of osteoporosis risk in patients with infertility ($p < 0.01$), however it was not possible to determine a similar relationship in the control group. The highest deficiencies in bone tissue density were demonstrated in the groups of the patients with lowered bioavailable testosterone level ($\text{bio-T} \leq 11.6 \text{ nmol/l}$) ($p < 0.05$). Moreover, the same study revealed a positive correlation between the concentration of 25(OH)D and spermatozoa motility and morphology in all the patients, irrespective of an initial type of semen disorders ($p < 0.05$). In the control group, this correlation did not reach statistical significance, but was also high (motility $p = 0.047$; morphology: $p = 0.056$). No correlation between concentrations of testosterone and vitamin D was observed in the above study (Yang B, 2012).

Hammoud et al. analyzed environmental and hormonal factors in 147 healthy men. This group was selected from 170 volunteers, so that it was uniform as regards an age (29 ± 8.5 years), BMI (24.3 ± 3.2) and stimulants intake, nicotinism, alcohol consumption. This study demonstrated unequivocally that both low ($< 20 \text{ ng/ml}$) and high ($> 50 \text{ ng/ml}$) concentration of vitamin D in serum negatively affected spermatozoa number per ml of semen, their

progressive movement and morphology (Hammoud , 2012). Similar conclusions were also drawn by the researchers from Denmark, who evaluated semen parameters in 307 young men with negative interview towards infertility. Not only poorer semen parameters, but also lower concentration of androgens in serum was demonstrated in the group with low 25(OH)D concentration (Fig. 3). As emphasized by the authors, this study cannot be the basis for unequivocal conclusions drawing due to low number of patients with low 25(OH)D concentration in the observation (Ramlau-Hansen, 2011). The results deaden an enthusiasm for uncritical supplementation with vitamin D, which except positive aspects may be also harmful.

Vitamin D effect on in vitro fertilization.

There are numerous studies analyzing an influence of vitamin D on success of in vitro fertilization (IVF) available in the current literature. Most of researchers compared the concentration of calciferol in serum and follicular fluid. Ozkan et al. (2010) examined 84 women treated using IVF method. The concentration of vitamin D in follicular fluid well correlated with its serum concentration ($p=0.001$) and was inversely proportional to BMI of the patients ($p=0.035$). Statistically significantly higher ($p=0.001$) concentration of vitamin D in follicular fluid was demonstrated in women of Caucasian race (30.51 ± 12.95 ng/ml) compared to blacks (18.88 ± 8.5 ng/ml). In the final analysis, the concentration of vitamin D in the patients who got pregnant (30.95%) was considerably higher ($p=0.013$) compared to these in which the pregnancy was not achieved. The patients with an initial concentration of vitamin D on an average level of 43.01 ± 10.65 ng/dl had four-time higher chance ($p=0.024$) for successful IVF compared to these with the lowest initial concentrations (16.74 ± 3.38). Similar results were obtained by Rudick et al. (2012) who evaluated serum vitamin D concentration in 188 patients treated in in vitro fertilization programme. A considerable difference between vitamin D concentrations in the examined population was demonstrated between the women

of Caucasian and Asian race ($p=0.001$). In Caucasian population, the chance of pregnancy achieving increased with vitamin D concentration, while in Asians the reverse relationship was demonstrated. Also this study, after an analysis taking into account the number and quality of transferred embryos, demonstrated that the patients with proper concentration of vitamin D have 4-time higher chance for successful IVF. Not all studies confirm the results described above. Firouzabadi et al. (2013) examined concentration of 25(OH)D in serum and follicular fluid in 221 patients treated due to infertility. In 22.6% of the patients, the concentration was <10 ng/dl, in 70.1% it was within the range 10-29 ng/dl, while in the remaining 7.2% it was higher than 30 ng/dl. Comparison of the groups, both in terms of fertilization percentage (43.17, 53.37, 58.77 %, respectively) and implantation (17.33, 15.26, 18.75 %, respectively), did not demonstrate any significant correlation ($p>0.5$). The analysis conducted did not demonstrate statistical significance ($p=0.170$) nor between vitamin D concentration in serum ($p=0.094$) or in follicular fluid, and IVF results.

Significant differences as a response on ovulation stimulation, number and quality of embryos depending on vitamin D concentration were not observed in none of the described research. Ruddick and Ozkan suggest that improved IVF results are related to profitable effect of vitamin D on endometrium (Ozkan, 2010; Ruddick, 2012). This mechanism was already examined and described by Evans in 2006 in Biology of Reproduction (Evans et al., 2006). It is known that 1,25(OH)₂D exhibits strong immunomodulating activity both towards lymphocytes T and the cells presenting antigen. Even decreased occurrence of autoimmune diseases and regression of symptoms in individuals stimulated with high doses of vitamin D was proved in the study on animals (Van Etten, 2003). High concentration of vitamin D and its metabolites was noted in human in decidua collected in the 1st trimester of pregnancy which suggests its contribution in proper implantation and local immunological preference of the embryo. Evans et al. in experiment in vitro on primary cultures of decidua

cells collected in the 1st and 3rd trimester evaluated an expression and activity of 1 α -hydroxylase (CYP27B1), enzyme catalyzing synthesis of 1,25(OH)₂D. It was demonstrated that the synthesis of vitamin D is higher in the cells collected in the 1st trimester of pregnancy (41 \pm 11.8 fmole/h/mg protein) compared to these from 3rd trimester (8 \pm 4.4 fmole/h/mg protein, $p < 0.05$). Also quantitative analysis RT-PCR demonstrated higher gene expression for *CYP27B1* both in the stromal cells CD10^{+VE} and CD10^{-VE} in the 1st trimester of pregnancy. Immunomodulating role of vitamin D was also confirmed by stimulation of NK cells (Natural Killer CD56^{+ve}) isolated from 1st trimester decidua. These cells, after 28 hours of incubation with 1,25(OH)₂D or 25(OH)D, demonstrated decreased production of cytokine MG CSF2 (granulocytes and macrophages colony stimulating factor), interleukin 6 and Tumor Necrosis Factor (TNF), and an increase in mRNA expression for Cathelicidin Antimicrobial Peptide (CAP), having a direct antibacterial activity (Evans et al., 2006). According to the authors, the results of the study prove an accelerated production of active form of vitamin D in decidua cells in the first trimester, which in a paracrine manner modulates reactions between mother's immune system and an embryo. Concentration of vitamin D in the first trimester of pregnancy was also evaluated as the marker of preeclampsia. However, not all opinions are compliant in this range. The study of Bomba-Opoń et al. (2013) which included 280 pregnant women did not demonstrate correlations between concentrations of 25(OH)D and this complication. We cannot be sure currently whether it is the only capture point for vitamin D activity in reproduction processes.

Further publications demonstrate quite different results. For example, the data of Anifandis et al. are interesting; they examined follicular fluid of 101 patients treated with IVF-ICSI method and determined concentration of both 1,25(OH)₂D and glucose (Anifandis et al., 2010). Poorer quality embryos ($p = 0.009$) and lower percentage of pregnancies (14.5%) was obtained in the group of patients in which vitamin D concentration was over 30 ng/dl,

compared to the group of patients with concentration in the range of 20.1-30 ng/dl (32.7%) and the patients in which vitamin D concentration was below 20 ng/dl (32.3%), ($p=0.047$). The patients with the highest concentrations of $1,25(\text{OH})_2\text{D}$ were concurrently characterized by the lowest glucose concentration in follicular fluid ($p=0.003$).

Obtaining of such different results is presumably caused by relatively small groups of women included in particular protocols. Holistic evaluation of vitamin D activity mechanism on fertility requires carefully planned cohort research conducted on sufficiently large population of the patients, especially that numerous scientific associations recommend vitamin D supplementation during pregnancy and lactation in amount of 800-1000 IU/day (Poreba et al. 2011). The guidelines of supplementation with vitamin D for all age groups of Middle European population were elaborated during the conference “Vitamin D – minimum, maximum, optimum”, which was held in Warsaw in October 2012, with a contribution of international experts from various fields (Pludowski et al., 2013). It was accepted that the treatment requires vitamin D concentration below 20 ng/ml (up to 50 nmol/l). The doses for adult and elderly people of proper body weight and pregnant and lactating women should not exceed 4000 IU/day (100 $\mu\text{g}/\text{day}$). The supplementation recommended in pregnant women is 1500-2000 IU/day (37.5-50.0 $\mu\text{g}/\text{day}$).

Summary

The results of the studies presented point an effect of vitamin D on fertility, both in women and men. The significance of vitamin D deficiency is emphasized, but also, however considerably more rarely, its excess in etiology of some hormonal disturbances and fertility disorders related to them. The study conducted point the need of vitamin D supplementation considering in infertility therapy in both partners. Administration of vitamin D is recommended in case when its considerable deficiency may be expected, especially in obese

women, these with insulin resistance and small ovarian reserve and in men with oligo- and asthenozoospermia.

References:

1. Mellanby E. An experimental investigation of rickets. *Lancet* 1919;1:407-412.
2. Troszyński M. Ćwiczenia Położnicze (Obstetric textbok – in Polish). PZWL Warszawa 2009
3. Seremak-Mrozikiewicz A, Drews K, Mrozikiewicz PM, Bartkowiak-Wieczorek J, Marcinkowska M, Wawrzyniak A, Slomski R, Kalak R, Czerny B, Horst-Sikorska W. Correlation of vitamin D receptor gene (VDR) polymorphism with osteoporotic changes in Polish postmenopausal women. *Neuro Endocrinol Lett.* 2009;30(4):540-6.
4. Prosser DE, Jones G. Enzymes involved in the activation and inactivation of vitamin D. *Trends Biochem Sci.* 2004 Dec;29(12):664-73.
5. Rosen CJ, Abrams SA, Aloia JF, Brannon PM, Clinton SK, Durazo-Arvizu RA, Gallagher JC, Gallo RL, Jones G, Kovacs CS, Manson JE, Mayne ST, Ross AC, Shapses SA, Taylor CL. IOM committee members respond to Endocrine Society vitamin D guideline. *Clin Endocrinol Metab.* 2012 Apr;97(4):1146-52.
6. Christakos S, Dhawan P, Benn B, Porta A, Hediger M, Oh GT, Jeung EB, Zhong Y, Ajibade D, Dhawan K, Joshi S. Vitamin D: molecular mechanism of action. *Ann N Y Acad Sci.* 2007 Nov;1116:340-8.
7. Luk J, Weisman Y, Harell A, Edelstein S, David M, Spierer Z, Golander A. 1 alpha, 25-Dihydroxyvitamin D3 and 24,25-dihydroxyvitamin D3 in vitro synthesis by human decidua and placenta. *Nature.* 1979 Sep 27;281(5729):317-9.
8. K Evans, L Nguyen, J Chan, B A. Innes, J N. Bulmer, M Kilby, M Hewison, Effects of 25-Hydroxyvitamin D3 and 1,25-Dihydroxyvitamin D3 on Cytokine Production by Human Decidual Cells, *Biology of Reproduction* December 1, 2006 vol. 75 no. 6 816-822
The Rotterdam ESHRE/ASRM-sponsored PCOS Consensus Workshop Group 2004 Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). *Hum Reprod* 19:41–47
9. Pérez-Fernandez R, Alonso M, Segura C, Muñoz I, García-Caballero T, Diguez C. Vitamin D receptor gene expression in human pituitary gland. *Life Sci.* 1997;60(1):35-42.
10. Parikh G, Varadinova M, Suwandhi P, Araki T, Rosenwaks Z, Poretsky L, Seto-Young D. Vitamin D regulates steroidogenesis and insulin-like growth factor binding protein-1 (IGFBP-1) production in human ovarian cells. *Horm Metab Res.* 2010 Sep;42(10):754-7.

11. Torrealday S, Neal Perry G, Pal L. Relevance of vitamin D in reproduction. *Hum Reprod.* 2012 Oct;27(10):3015-27
12. Aquila S, Guido C, Middea E, Perrotta I, Bruno R, Pellegrino M, Andò S. Human male gamete endocrinology: 1 α , 25-dihydroxyvitamin D₃ (1,25(OH)₂D₃) regulates different aspects of human sperm biology and metabolism. *Reprod Biol Endocrinol.* 2009.
13. Habib FK, Maddy SQ, Gelly KJ. Characterisation of receptors for 1,25-dihydroxyvitamin D₃ in the human testis. *J Steroid Biochem.* 1990 Feb;35(2):195-9.
14. Azziz R, Carmina E, Dewailly D, Diamanti-Kandarakis E, Escobar-Morreale HF, Futterweit W, et al. Criteria for Defining Polycystic Ovary Syndrome as a Predominantly Hyperandrogenic Syndrome: An Androgen Excess Society Guideline. *J Clin Endocrinol Metab* 2006;91:4237-4245
15. Ricardo Azziz, Diagnosis of Polycystic Ovarian Syndrome: The Rotterdam Criteria Are Premature, *The Journal of Clinical Endocrinology & Metabolism* March 1, 2006 vol. 91 no. 3 781-785
16. Thomson RL, Spedding S, Buckley JD. Vitamin D in the aetiology and management of polycystic ovary syndrome. *Clin Endocrinol (Oxf).* 2012 Sep;77(3):343-50
17. Patra SK, Nasrat H, Goswami B, Jain A. Vitamin D as a predictor of insulin resistance in polycystic ovarian syndrome. *Diabetes Metab Syndr.* 2012 Jul-Sep;6(3):146-9.
18. Ott J, Wattar L, Kurz C, et. all. Parameters for calcium metabolism in women with polycystic ovary syndrome who undergo clomiphene citrate stimulation: a prospective cohort study *Eur J Endocrinol* May 1, 2012 166 897-902
19. Wehr E, Pilz S, Schweighofer N, Giuliani A, Kopera D, Pieber TR, Obermayer-Pietsch B. Association of hypovitaminosis D with metabolic disturbances in polycystic ovary syndrome. *Eur J Endocrinol.* 2009 Oct;161(4):575-82
20. Yildizhan R, Kurdoglu M, Adali E, Kulusari A, Yildizhan B, Sahin HG, Kamaci M. Serum 25-hydroxyvitamin D concentrations in obese and non-obese women with polycystic ovary syndrome. *Arch Gynecol Obstet.* 2009 Oct;280(4):559-63.
21. Christakos S, Hewison M, Gardner DG, Wagner CL, Sergeev IN, Rutten E, Pittas AG, Boland R, Ferrucci L, Bikle DD. Vitamin D: beyond bone. *Ann N Y Acad Sci.* 2013 May;1287:45-58.
22. Nestler JE, Reilly ER, Cheang KI, Bachmann LM, Downs RW Jr. A pilot study: effects of decreasing serum insulin with diazoxide on vitamin D levels in obese women with polycystic ovary syndrome. *Trans Am Clin Climatol Assoc.* 2012;123:209-19; discussion 219-20

23. Paffoni A, Somigliana E, Vigano P, Benaglia L, Cardellicchio L, Pagliardini L, Papaleo E, Candiani M, Fedele L. Vitamin D status in women with uterine leiomyomas. *J Clin Endocrinol Metab.* 2013 Aug;98(8):E1374-8
24. Di Rosa M, Malaguarnera G, De Gregorio C, Palumbo M, Nunnari G, Malaguarnera L. Immuno-modulatory effects of vitamin D3 in human monocyte and macrophages. *Cell Immunol.* 2012 Nov;280(1):36-43
25. Somigliana E, Panina-Bordignon P, Murone S, Di Lucia P, Vercellini P, Vigano P. Vitamin D reserve is higher in women with endometriosis. *Hum Reprod.* 2007 Aug;22(8):2273-8
26. Jenny A Visser, Frank H de Jong, Joop S E Laven, Axel P N Themmen Anti-Müllerian hormone: a new marker for ovarian function, *Reproduction* January 1, 2006 131 1-9
27. Kelsey TW, Wright P, Nelson SM, Anderson RA, Wallace WHB (2011). "A validated model of serum anti-Müllerian hormone from conception to menopause". In Vitzthum, Virginia J. *PLoS ONE* 6 (7): July 2011
28. Seifer DB, Maclaughlin DT Review Mullerian Inhibiting Substance is an ovarian growth factor of emerging clinical significance. *Fertil Steril.* 2007 Sep; 88(3):539-46
29. Malloy PJ, Peng L, Wang J, Feldman D Interaction of the vitamin D receptor with a vitamin D response element in the Mullerian-inhibiting substance (MIS) promoter: regulation of MIS expression by calcitriol in prostate cancer cells. *Endocrinology.* 2009 Apr; 150(4):1580-7.
30. Merhi ZO, Seifer DB, Weedon J, Adeyemi O, Holman S, Anastos K, Golub ET, Young M, Karim R, Greenblatt R, Minkoff H. Circulating vitamin D correlates with serum antimüllerian hormone levels in late-reproductive-aged women: Women's InteragencyHIV Study. *Fertil Steril.* 2012 Jul;98(1):228-34
31. Yu HM, Li XJ, Kadam AL, Cheng CY, Koide SS. Human testis vitamin D Winding protein involved in infertility. *Arch Androl.* 1994 Sep-Oct;33(2):119-28
32. Yang B, Sun H, Wan Y, Wang H, Qin W, Yang L, Zhao H, Yuan J, Yao B. Associations between testosterone, bone mineral density, vitamin D and semen quality in fertile and infertile Chinese men. *Int J Androl.* 2012 Dec;35(6):783-92
33. Hammoud AO, Meikle AW, Peterson CM, Stanford J, Gibson M, Carrell DT. Association of 25-hydroxy-vitamin D levels with semen and hormonal parameters. *Asian J Androl.* 2012 Nov;14(6):855-9
34. Ramlau-Hansen CH, Moeller UK, Bonde JP, Olsen J, Thulstrup AM. Are serum levels of vitamin D associated with semen quality? Results from a cross-sectional study in young healthy men. *Fertil Steril.* 2011 Mar 1;95(3):1000-4

35. Ozkan S, Jindal S, Greenseid K, Shu J, Zeitlian G, Hickmon C, Pal L. Replete vitamin D stores predict reproductive success following in vitro fertilization. *Fertil Steril*. 2010 Sep;94(4):1314-9.
36. Rudick B, Ingles S, Chung K, Stanczyk F, Paulson R, Bendikson K. Characterizing the influence of vitamin D levels on IVF outcomes. *Hum Reprod*. 2012 Nov;27(11):3321-7.
37. Firouzabadi RD, Rahmani E, Rahsepar M, Firouzabadi MM. Value of follicular fluid vitamin D in predicting the pregnancy rate in an IVF program. *Arch Gynecol Obstet*. 2013 Jul 24. [Epub ahead of print]
38. Van Etten E, Decallonne B, Verlinden L, Verstuyf A, Bouillon R, Mathieu C. Analogs of 1alpha,25-dihydroxyvitamin D3 as pluripotent immunomodulators. *J Cell Biochem*. 2003 Feb 1;88(2):223-6)
39. Bomba-Opon D, Brawura-Biskupski-Samaha R, Kozłowski S, Kosinski P, Bartoszewicz Z, Bednarczuk T, Wielgos M. First trimester maternal serum vitamin D and markers of preeclampsia. *J Matern Fetal Neonatal Med*. 2013 Sep [Epub ahead of print] PubMed PMID: 24050181
40. Anifandis GM, Dafopoulos K, Messini CI, Chalvatzas N, Liakos N, Pournaras S, Messinis IE. Prognostic value of follicular fluid 25-OH vitamin D and glucose levels in the IVF outcome. *Reprod Biol Endocrinol*. 2010 Jul 28;8:91
41. Poreba R, Drews K, Karowicz-Bilińska A, Oszukowski P, Pawelczyk L, Radowicki S, Spaczyński M, Szczapa J; Zespołu Ekspertów Polskiego Towarzystwa Ginekologicznego. [Expert review of Polish Gynecological Society regarding micronutrient supplementation in pregnancy]. *Ginekol Pol*. 2011 Jul;82(7):550-3
42. Płudowski P, Karczmarkiewicz E, Bayer M, Carter G et al. Practical guidelines for the supplementation of vitamin D and the treatment of deficits in Central Europe – recommended vitamin D intakes in general population and group at risk of vitamin D deficiency. *Endokrynol Pol* 2013: 64(4):319-327.

Figures:

Figure 1 - The role of vitamin D in the reproductive system

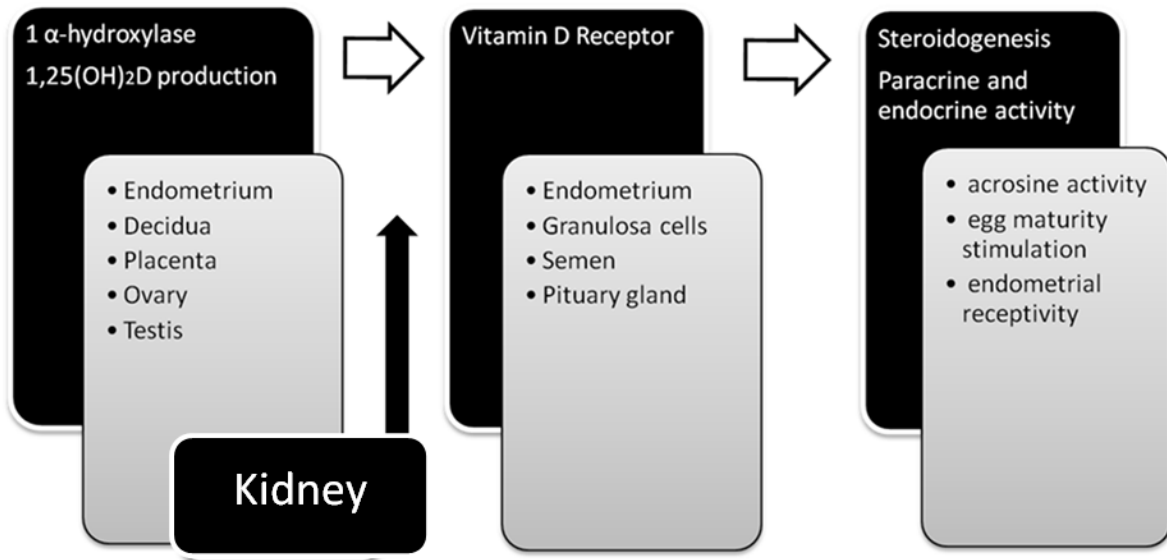


Figure 2 - The effects and causes of vitamin D deficiency in women with PCOS

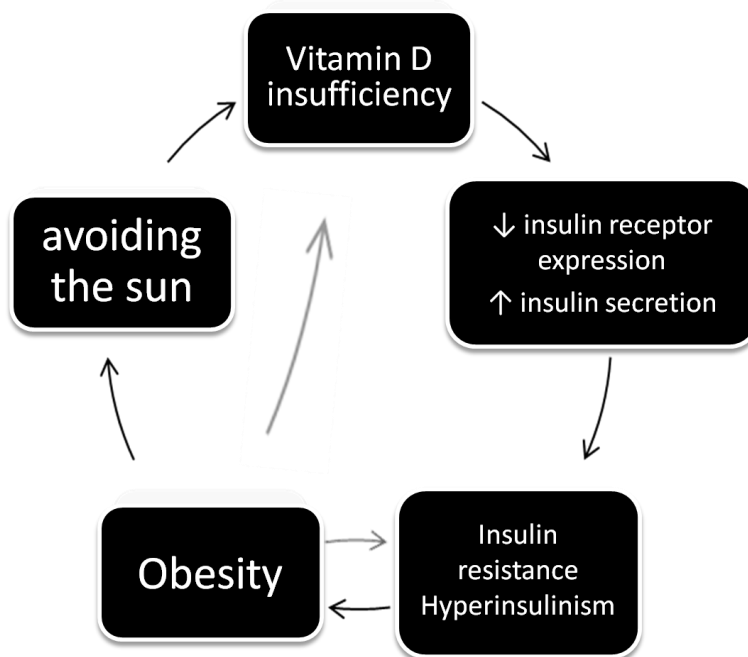


Figure 3 - The influence of vitamin D on the activity and the morphology of sperm



>50ng/ml

reduced in number and motility of sperm, ↑ morphology defects

<20ng/ml

