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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 60 377 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 D_2 (ergocalciferol) and D_3 (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 D_3 is contained in sea fish fat and cod liver oil, while D_2 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 it 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 (Figure 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.

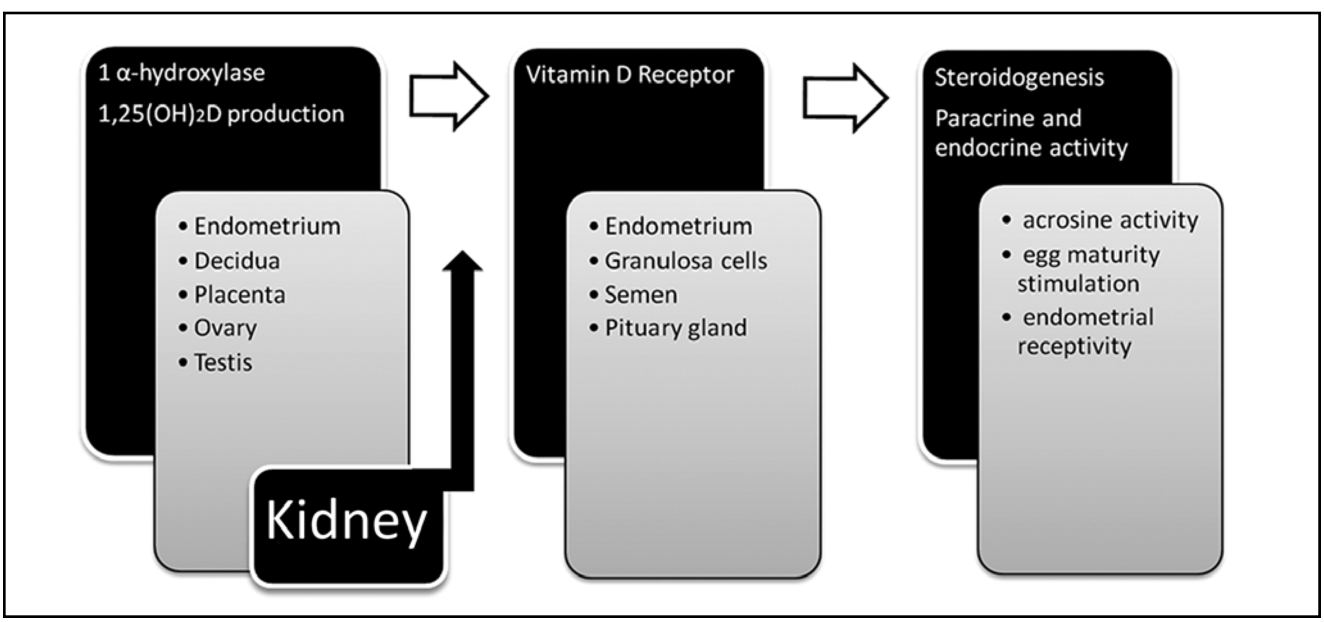

Fig. 1. The role of vitamin D in the reproductive system.

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.

RESULTS

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

<u>*Polycystic ovary syndrome*</u>

PCOS is the most often diagnosed endocrinological disorder in women in reproductive period. It is recognized based on Rotterdam criteria or according to Androgen Access 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 *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 (Figure 2). Vitamin D affects glucose transport to the cell via stimulation of an expression of insulin receptor and insulin secretion (Christakos 2013).

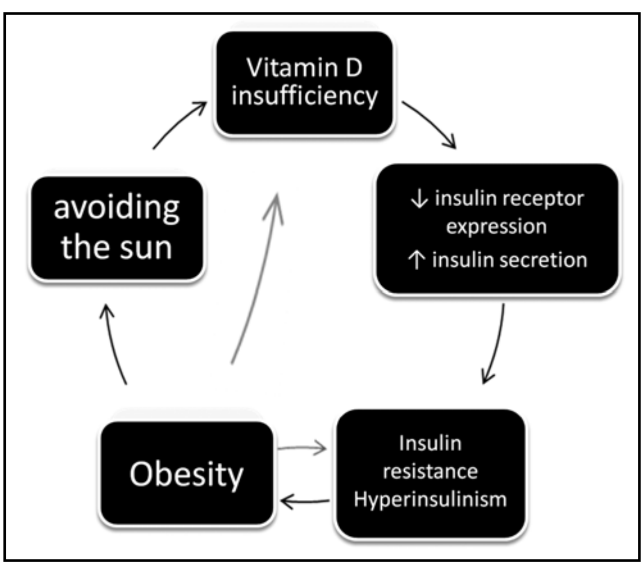

Fig. 2. The effects and causes of vitamin D deficiency in women with PCOS.

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).

<u>*Uterine myomas*</u>

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 $25(OH)D_3$, $1,25(OH)_2D_3$, 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 28.2 ng/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, $1,25(OH)_2D$ 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 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 boned 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 (bio-T ≤11.6 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 2012).

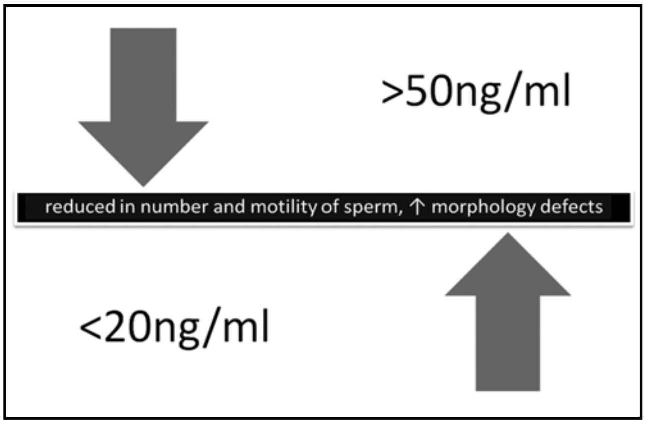

Fig. 3. The influence of vitamin D on the activity and the morphology of sperm.

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 ng/ml) and high (>50 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 (Figure 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)$_2$D exhibits strong immunomodulating activity both towards lymphocytes T and the cells presenting antigen. Even decreased occurrence of autoimmunological 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 1α-hydroxylase (CYP27B1), enzyme catalyzing synthesis of 1,25(OH)$_2$D). It was demonstrated that the synthesis of vitamin D is higher in the cells collected in the 1st trimester of pregnancy

(41±11.8 fmole/h/mg protein) compared to these from 3rd trimester (8±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 en 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 $1,25(OH)_2D$ 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 (Poręba *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(Płudowski *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 µg/day). The supplementation recommended in pregnant women is 1500–2000 IU/day (37.5–50.0 µg/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.

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