Introduction

In recent years, there has been a growing interest in studying the association of vitamin D deficiency and infertility. It has been postulated that vitamin D receptors (VDR) are found in human tissues such as male and female reproductive organs and play a major role in facilitating the biological activity of Vitamin D. Vitamin D deficiency has been advocated as a possible cause of infertility in many studies conducted in the past several years. This review article aims to systematically review the studies associating the role of Vitamin D in infertility from 2004 to 2017 and analyses their findings and limitations associated with the study.

A systematic search of scientific literature from published studies evaluating the role of vitamin D in infertility was performed in electronic databases from year 2004 to 2017. The articles included both prospective and retrospective studies available on the net.

Many researchers have studied the role of vitamin D and its association with reproductive health extensively in the last few years but there is no single consensus on its influence in reproductive health. While it is a general observation that optimal level of vitamin D is essential in PCOS, endometriosis, male infertility and IVF techniques, but there has been no significant correlation between vitamin D levels and ovulation stimulation or embryo development and Vitamin D levels. However, larger studies including all ethnic and racial groups would be required to proclaim the role of Vitamin D in infertility.

Vitamin D, also known as “sunshine hormone”, is a fat soluble hormone which plays an integral part in calcium and phosphorous homeostasis and maintainence of healthy bones and teeth and is involved in providing protection against a number of diseases such as cancer, diabetes, multiple sclerosis, cardiovascular diseases, obesity and many other diseases including its role in infertility [1-6].

Vitamin D is considered to be a prohormone and is synthesized by skin on exposure to sunlight as Vitamin D3 or cholecalciferol. Vitamin D2 or ergoclaciferol is obtained from yeast and dietary sources. Vitamin D deficiency can result from inadequate exposure to sunlight, malabsorption syndromes and certain drugs like dilantin, phenobarbitol and rifampicin

which induce hepatic P450 enzymes to accelerate the catabolism of vitamin D [7].

In recent years, there has been a growing interest in studying the association of vitamin D deficiency and infertility [8-10]. It has been postulated that vitamin D receptors (VDR) are found in human tissues such as male and female reproductive organs and play a major role in facilitating the biological activity of Vitamin D [1-3] In the US, it has been estimated that about one third of the population is deficient in Vitamin D and infertility affects nearly 15.5% of the US couples and nearly 53 million people all across the globe [11,12]. Vitamin D deficiency has been advocated as a possible cause of infertility in many studies conducted in the past several years.

This review article aims to systematically review the studies associating the role of Vitamin D in infertility from 2004 to 2017 and analyse their findings and limitations associated with the study.

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The study conducted by Corbett et al. in 2004 [13] in New Hampshire USA aimed to investigate the presence of VDR in human sperm. Semen samples from 11 fertile and 20 infertile men were analysed. They concluded that VDR is present in the midpiece of human sperm and VDR expression was inversely proportional to sperm concentration in infertile men as compared to fertile controls. They observed a downward trend in VDR expression for patients with low motility irrespective of fertile status. The study was limited by small sample size [13]. In a further study conducted in 2006, they analysed semen samples from 10 fertile men and observed that VDR is expressed in mainly head/nucleus in the post acrosome portion and mid piece of sperms in all fertile men. Their study excluded all infertile men due to low or undetectable VDR expression or oligospermia, low motility and poor morphology [14]. Their study was limited by small sample size and inclusion of only fertile men.

In 2010, Anifandis et al. carried out a prospective study in Greece to measure

serum and follicular fluid (FF) 25(OH) D and glucose levels in women who underwent IVF or embryo transfer to evaluate the success rate of IVF in 101 women. (8)They observed that FF 25(OH) D had a significant correlation with embryo quality (R=0.27, P=0.027) and that FF glucose levels were lower with high Vitamin D (p-0.003). Clinically, pregnancy rates were lower with high vitamin D (P=0.047) in their study. They observed that high vitamin D and low FF glucose have a negative effect on embryo quality and IVF outcomes.

The first comprehensive analysis of the potential role of vitamin D in male reproduction was carried out by Bloomberg et al in Denmark in 2010. They analysed 13 men with orchidectomies due testicular cancer and/or prostatectomies and concluded that there was marked expression of VDR and Vitamin D metabolizing enzymes in human testis, ejaculatory tract and mature spermatozoa suggesting that Vitamin D plays a pivotal role in spermatogenesis and maturation of human sperms. [3]. However, their study was limited by the fact that they carried out their study on pathological specimens.

Ozkan et al. [5] carried out a prospective study in New York in 2010 to determine the outcome of IVF cycles in relation to Vitamin D levels in the FF of 84 infertile women. They too found a significant correlation between serum and FF vitamin D (R=0.94). They observed that women with higher Vitamin D levels were more likely to have positive outcome of IVF (P=0.013). They concluded that Vitamin D supplementation given to deficient subjects could improve fertility outcomes [5].

Ramlau et al. conducted a crosssectional study in 2011 in Denmark in 347 young men to examine the association between low serum Vitamin D and male reproductive function [15]. They observed that a high Vitamin D level was unexpectedly associated with lower crude median total sperm count and percentage of sperms with normal morphology and a high level of crude sex hormone binding globulin and FSH. Men with high level of vitamin D had 11% lower free androgen index as compared to men with adequate Vitamin D. Their study did not find any correlation between low Vitamin D levels and poor semen quality.

Bloomberg et al. in their study in 2011 analysed 300 young men for semen quality and Vitamin D levels to assess the correlation between the two. They observed a positive correlation between sperm motility and progressive motility with Vitamin D levels. ($p<0.05$) Men with lower vitamin D levels had lower proportion of motile ($p=0.027$), progressively motile ($p-=0.035$) and morphologically normal spermatozoa ($p=0.004$) as compared to men with high vitamin D levels. They further observed that activated Vitamin D increased intracellular calcium concentration, sperm motility and induced acrosomal reaction in mature sperms suggesting a role of vitamin D in optimal sperm function [4]. However, their study was limited by the fact that only fertile men were included in the study.

Similar findings were observed by Aleyasin et al. [1] in their prospective cohort study in 2011 in Asian men where they found a positive correlation between vitamin D levels in serum and FF ($R=0.767$, $p=0.001$). They observed that fertilization rated decreased significantly ($p=0.018$) and implantation rates increased ($p=0.791$) with higher vitamin D levels. Their observation was contrary to the observations made by other researchers. They concluded that low vitamin D has no correlation with outcome of ART [1].

In a retrospective study carried out by Li et al in California in 2012 in 1192 women of reproductive age, it was observed that majority of the infertile women had low vitamin D levels with 68.6% women having levels <32 ng/ml and 22.2% having <20 ng/ml levels. They observed that high BMI, Asian and blacks were all at high risk of vitamin D deficiency, particularly Asian women [9]. Similarly, Rainer et al in 2012 in Oklahoma retrospectively evaluated 53 PCOS women and found that vitamin D deficiency was highly prevalent in PCOS women. However, they did not find any positive correlation with levels of Vitamin D and time to pregnancy [10].

Rudick et al. in 2012 [6], in their retrospective study, aimed to validate the findings by other investigators in 188 fertile women undergoing IVF and observed that low vitamin D is associated with lower pregnancy rates in Hispanic whites ($p=0.04$) but not in Asians. They observed that Asians had lower pregnancy rates with low vitamin D levels ($p=0.01$) and that Vitamin D

was lower in younger (p=0.01) and heavier (p=0.03) women and had diminished ovarian reserve (p=0.01). The reverse correlation observed in their study could be due to small sample size of Asians [6].

In 2013, Garbedian et al. [17] in Canada classified 173 women aged 18-41 years into white, black and other categories to investigate the role of Vitamin D in IVF patients. Their observation was that optimal Vitamin D levels had higher clinical outcomes of IVF (52.5%) as compared to women with low vitamin D (34.7%). However, implantation rate in both the groups was not statistically significant (p=0.6) [17].

In a prospective cross-sectional study in Italy by Paffoni et al. in 2014 to investigate IVF outcomes and Vitamin D levels in 154 Caucasian women, clinical pregnancy were 20% in women with vitamin D<20 ng/ml and 31% in women with vitamin D> 20 ng/ml (p=0.02) [18].

Rudick et al. [16] in 2014 found that Vitamin D deficient Caucasian females had lower clinical pregnancy rates as compared to Vitamin D adequate recipients (37% vs. 78%). They found that live birth rate was 31%among Vitamin D deficient subjects as compared to 59% among Vitamin D sufficient subjects. They concluded that vitamin D may be mediated through the endometrium [16]. However, Firouzabadi et al. [2] in 2014 did not find any significant correlation between pregnancy rate and serum Vitamin D levels (p=0.094) or the FF vitamin D level (p=0.170). The serum Vitamin D and FF vitamin D levels correlated significantly (p=0.000) in their study [2].

Abbasihormozi et al. [19] in 2017 published a study about the association of Vitamin D status with semen quality and reproductive hormones in Iranian sub fertile men. They observed that in normospermic men, serum vitamin D levels did not correlate with semen parameters and reproductive hormones, whereas there was a positive correlation with vitamin D in oligoasthenotetrazoospermic men (r=0.131, p=0.028) [19].

The role of vitamin D and its association with reproductive health has been studied extensively by many researchers in the last few years but still there is no single consensus on its influence in reproductive health. While it is a general observation that optimal level of vitamin D is essential fin PCOS,

endometriosis, male infertility and IVF techniques, but there has been no significant correlation between vitamin D levels and ovulation stimulation or embryo development and Vitamin D levels. However, larger studies including all ethnic and racial groups would be required to proclaim he role of Vitamin D in infertility.

High Prevalence of Vitamin D Deficiency in Infertile Women Referring for Assisted Reproduction

Animal and human studies suggest that vitamin D is involved in many processes of the human reproductive system in both genders. Of those, some evidence come from the Assisted Reproduction Technology (ART) [20,21]. ART represents a valuable model to draw inferences on vitamin D deficiency in specific aspects of human fertility as it allows the separate evaluation of the various steps of the reproductive process, from sperm function to folliculogenesis to embryo implantation.

Six original articles have investigated the association between serum levels of 25-hydroxy-vitamin D (25(OH)D), the storage form of the vitamin, and pregnancy rates in ART cycles with controversial results. For instance, Rudick *et al*. [22] observed that serum 25(OH)D levels were significantly related to implantation, clinical pregnancy, and live birth rates, although opposite trends were found according to patients' ethnicity being critical in non-Hispanic whites, but not in the Asian ethnicity. In a second study , the same authors examined serum 25(OH)D concentration among recipients of oocyte donation, finding a positive association between vitamin D status and clinical pregnancy rate and suggesting the specific effect of 25(OH)D levels on ART outcomes to be mediated by endometrial receptivity rather than by ovarian stimulation or embryo parameters. To this regard, it should be emphasized that both cyclic and early pregnant endometrium represents an extra-renal site of vitamin D synthesis; moreover, the effect of vitamin D at the uterine level is thought to be exerted via the vitamin D receptor (VDR) through either the regulation of target genes or the hormonal effects on the local immune responses [32]. In patients who underwent single embryo transfer at blastocyst stage, vitamin D deficiency (<20 ng/mL) emerged as an independent predictor of lower clinical pregnancy rates as compared with non-

deficient women [25]. In contrast, in the largest sample analyzed so far, Franasiak *et al*. [26] showed that vitamin D status was unrelated to pregnancy outcomes in women undergoing euploid blastocyst transfer. Two Iranian studies did not confirm any influence of serum 25(OH)D levels in terms of pregnancy rate [27,28*et al*. reported a correlation between follicular fluid 25(OH)D concentration and assisted reproductive outcomes in an Iranian population [29].

Results from a further cross-sectional prospective study supported the role of vitamin D in terms of female fertility [20]; indeed the odds ratio (OR) for clinical pregnancy in women with vitamin D greater than 20 ng/mL was 2.15 (95% CI 1.23–3.77). Likewise, the group with serum levels >30 ng/mL (sufficient vitamin D) had the highest chances of pregnancy. A similar figure was observed when considering the implantation rate (OR 1.91, 95% CI 1.20–3.05). Finally, women with sufficient 25(OH)D concentration had a significantly higher chance of obtaining top quality embryos and to transfer at the blastocyst stage, thus generally supporting a favorable effect of vitamin D at both ovarian and endometrial level [20].

Based on these observations, we comprehensively analyzed vitamin D status of couples attending a single academic infertile center. Such an evaluation would provide an estimation of a cross-sectional epidemiological magnitude of vitamin-D deficiency, while forming the basis for interventions to address the deleterious consequences of vitamin D deficiency-related infertility problems. We aimed to (i) determine the baseline vitamin D profiles of women attending an infertility center; and, (ii) investigate the non-dietary determinants of vitamin D status in the same cohort. We hypothesized that (i) a high proportion of our cohort would be vitamin D deficient; and (ii) vitamin D status in infertile women mainly relies on social habits, degree of sun exposure and health risk factors. This issue is also of particular clinical interest considering the growing and consistent evidence supporting the idea that vitamin D deficiency may influence birth outcomes and may be associated with relevant obstetrics complications [30,31,32,33].

vitamin D and ovarian

Vitamin D plays a crucial role in maintaining calcium homeostasis and bone density within the human body. Its biologic actions are mediated via the vitamin D receptor, most commonly found in calcium-regulating tissues such as the bones and parathyroid glands [1]. However, the identification of vitamin D receptors in reproductive organs such as the ovary, endometrium, testis, hypothalamus, and pituitary has fueled an ongoing interest about the role of vitamin D in human reproduction [34]. Prior studies have demonstrated that vitamin D can alter anti-müllerian hormone (AMH) signaling, follicle stimulating hormone (FSH) sensitivity and progesterone (P) production and release in human granulosa cells (34). Furthermore, a recent meta-analysis of women undergoing assisted reproduction reported that women replete in vitamin D have higher odds of live birth (odds ratio 1.33) when compared to those with deficient or insufficient vitamin D levels [35]. Given the almost epidemic pervasiveness of vitamin D deficiency among reproductive-age women, studies exploring the impact of vitamin D deficiency on ovarian physiology are of prime importance.

This issue features a retrospective cohort study by Shapiro et al. [36] that investigates the association between vitamin D levels and ovarian reserve parameters in a large cohort of infertile women. The study was conducted at an academically-affiliated private fertility center with a high prevalence of women with diminished ovarian reserve (DOR). Four-hundred-fifty-seven women between the ages 21 and 50 years were included. The women within this cohort had baseline vitamin D, specifically 25-hydroxyvitamin D (25OH-D), AMH, and FSH levels measured within 90 days of each other. Women were then sub-divided into two groups based on their age (i.e., <38 years or ≥ 38 years). Receiver operating characteristic curves were then created to test whether 25OH-D levels were predictive of AMH levels at 3 different threshold levels – 0.5 ng/mL, 1.0 ng/mL, and 5.0 ng/mL. The authors noted that 74 and 383 women were deficient and replete in 25OH-D, respectively, based on a <20 ng/mL cut-off. Interestingly, 25OH-D levels were comparable in both groups. The authors also noted that 25OH-D levels were poor predictors of AMH in both age sub-groups and across all AMH cut-offs. Furthermore, multivariate linear regression of log

transformed AMH and FSH, with 25OH-D levels adjusted for confounders indicated no correlation between 25OH-D and the measured ovarian reserve parameters.

Despite the retrospective nature of the study and its lack of information about race or ethnicity, the current study provides pertinent clinical data about vitamin D and ovarian reserve in a large cohort of infertile women with high prevalence of DOR. As indicated by the authors, the difference between the current results and prior studies may primarily be due to the inclusion of infertile women with DOR, as opposed to fertile women. It is encouraging to note that at least two independent studies in different patient populations have reported similar clinical results. In a retrospective study by Fabris et al. [37], the authors assessed the association between vitamin D levels and ovarian reserve, ovarian stimulation response and reproductive outcome in 851 donor oocyte cycles and found no correlation between vitamin D and AMH levels. In another prospective cross-sectional study, Drakopoulous et al. [38] included 283 infertile women <42 years undergoing their first assisted reproduction cycle; the authors reported no difference in mean AMH or antral follicle counts in women with normal or deficient vitamin D levels.

The collective results of these studies suggest that vitamin D levels are unlikely to have a direct impact on ovarian reserve parameters. Prospective studies that further elucidate the role of vitamin D in ovarian physiology and ovarian dysfunction are still required, especially in infertile women.

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