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Original Research Article

Relation between maternal vitamin D level and *in vitro*-fertilization laboratory outcome in normal responding patients 35 years old or less

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ABSTRACT

Background: Vitamin D relation to pregnancy outcome have been extensively studied and proved, but the relation between the vitamin D and fertility and fertility treatment especially in vitro-fertilization still a matter of debate.
Methods: A retrospective cohort study in large IVF center in Abu Dhabi, UAE involved young normal responding patients aged 35 years old or less attending the clinic for IVF treatment. Patient were divided in to three groups according to the serum level of vitamin D. the IVF laboratory outcome was calculated and compared in the three groups.
Results: We found that the prevalence of vitamin D in patients seeking fertility treatment especially IVF was significantly high. But on the other hand, and after analysing the results of the cycles laboratory out come in the three groups, we found that no significant difference that could be attributed to maternal level of vitamin D.
Conclusions: The prevalence of vitamin D deficiency and insufficiency in infertile patients is significantly high but no significant effect of vitamin D deficiency on the IVF laboratory outcome.

Keywords: Infertility, Vitamin D, *In vitro* fertilization

INTRODUCTION

Several factors have been shown to affect the *in vitro-fertilization* (IVF) cycles outcome either laboratory or clinically. The most important factors that could contribute to the success of IVF cycles were maternal age, ovarian reserve, previous cycles history, maternal and paternal chromosomal status, stimulation protocols, IVF laboratory experience, uterine abnormalities, endometrial receptivity, sperm parameters, grading and ploidy statues of the embryos.[1,2]

Finally and recently paternal age.[3] Some other factors could also contribute but to lesser extent such as maternal body mass index, maternal and paternal vitamin D level which both still a matter of debate.[4,5] Vitamin D receptors had been found in a lot of tissues all over the human body and It has been found that the triggering of vitamin D receptors could adjust directly or indirectly gene expression in huge number of tissues in the human body, hence its deficiency had been linked to a lot of chronic diseases such as obesity, cardiovascular, metabolic and immune diseases, this is beside its relation to malignancies, abnormal placentation and bad obstetric outcome such as pre-eclampsia, small for gestational age foetus, and increased risk for caesarean delivery.[6-8]

Vitamin D linked to the fertility due to expression of vitamin D receptors in the pituitary gland, ovarian tissue, granulosa cell, endometrium, myometrium, testis, epididymis, prostate, seminal vesicle beside its effect on sperm parameters and performance.[9] Vitamin D had also found to affect the level of anti-mullerian hormone, follicle stimulating hormone signalling and sensitivity which could affect granulosa cells, steroidogenesis and folliculo-genesis.[10,11] Vitamin D present in the serum in the form of vitamin D3 (cholecalciferol), which is synthetized in the

skin and it is assessed by measuring serum 25-hydroxy cholecalciferol level, if the level of vitamin D_3 is less than, 50 nmol/l considered deficient, between 50-75 nmol/l considered insufficient, and above than 75 nmol/l considered normal.[12,13] Deficiency of vitamin D results in low calcium blood level which leads to negative effect on oocyte maturation and development, on the other side calcium deficiency in males could also results in atheno-spermia and abnormal fertilization.[14,15]

Presence of vitamin D receptors in the endometrium and its antiproliferative, anti-inflammatory and immunoregulatory property affects myometrial contraction and cell proliferation and expression of homebox gene HOXA10 that is well known to alter implantation by promoting immunosuppression, extra-villous trophoblastic invasion and decidualization, it also regulates the production of human chorionic gonadotrophin, human placental lactogen, oestradiol and progesterone.[16-18]

Vitamin D through its immunosuppressive effect on the endometrium influence the uterine natural killer cells, macrophages and T-cells, with its anti-inflammatory and anti-migratory effect on the TH1 and TH2 cells resulting in promoting release of favourable cytokines that helps the implantation.[19] All this with abnormal implantation and placentation in patients who had low vitamin D level have raised the question that the abnormal fertility outcome in those patients could be related to endometrium and endometrial receptivity or to the performance of the gametes. Based on all these theories, several research have been done to find the effect of low vitamin D level on the IVF cycles outcome and weather the effect on the gametes and its performance or the endometrium and its receptivity.[20]

Some studies had succeeded to fined relation between the level of vitamin D and the outcome IVF cycles, while others failed to find any relation between both.[21,22] In our study and in order to evaluate the effect of vitamin D level on the performance of the gametes in IVF cycles we tried to unify other factors that could contribute to the outcome and we choose to evaluate the gamete performance as a laboratory IVF outcome to avoid possibility of involvement of vitamin D on the endometrium which later affect the clinical outcome.

METHODS

A retrospective cohort study that carried out in large IVF canter in Abu Dhabi (Fakih IVF) in period between January 2019-December 2019 and included infertile patients aged 35 years old or less and diagnosed as normal responders through AMH (1.2-4.9 ng/l) and antral follicle count with transvaginal scan on day 2-3 of her menstrual cycle.

Patients diagnosed as high or low responders on previous cycles were excluded, patients with known chromosomal abnormalities and couples with severely affected sperm parameters also were excluded. All patients had antagonist protocol. All patients counselled and consented for the IVF cycles.

Ovarian stimulation

Patients attended the clinic on their cycle day 2-3, transvaginal scan done to evaluate the ovaries and endometrium to ensure normal ovaries and complete endometrial shedding. Blood test was carried on for the basal ovarian hormones, vitamin D level. Stimulation starts with rFSH, and highly purified FSH/LH then we add gonadotrophin antagonist injections daily from day 6. Transvaginal scan to be done after one week to check ovarian response to stimulation and to decide for trigger injection timing. rHCG in a dose of 500 IU/l and to arrange for oocyte retrieval under sedation within 36 hours of trigger. Anaesthesia check-up to be done before the procedure.

On the day of oocyte retrieval, patients come fasting and under sedation ultrasound guided oocytes retrieval to be done, oocytes to be handed to the IVF laboratory for checking, denudation and insemination using the husband sperm which was previously prepared. Oocytes incubation then follow up for cleavage and blastocyst formation. On day 5 blastocyst evaluation carried out either for transfer, freezing and or tropho-ectoderm biopsy for preimplantation genetic diagnosis and screening.

In our study we divided patients included in this study into 3 groups according to their vitamin D level which were assist on the start of the IVF cycles, group 1 included patients with vitamin D level of 50 nmol/l (vitamin D deficiency group), between 50-75 nmol/l group 2 (vitamin D insufficient group), and group 3 included patients with vitamin D more than 75 nmol/l group. In all groups we evaluated the number of oocytes collected, number of mature oocytes, number of fertilized oocytes, number of cleaved oocytes and number of oocytes that reached the blastocyst stage. Also, we evaluated the maturation rate, cleavage rate, rate of blastocyst formation.

Statistical analysis

All the data well be presented in excel sheets with information presented as mean±standard deviations in tables. The significance will be calculated with the p value<0.05 using the chi square and one-way ANOVA test for independent variants calculators.

RESULTS

Our study included 273 cycles divided in to 3 groups according to vitamin d level, the mean age of all patients was 30±3.99, mean BMI was 27.67±5.48, mean AMH was 2.85±1.04, mean vitamin D level was 57.90±27.90 nmol/l. Group 1 with deficient vitamin d included 108 cases with mean age 30±3.64, mean BMI 28.55±5.48, mean AMH of

3.06±1.04, mean vitamin D level was 35.85±9.03 nmol/l. group 2 contains 103 cycles with mean age of 30±4.32, mean BMI 26.72±4.49, mean AMH of 2.74±0.97, mean vitamin D level was 60.73±6.24nmol/l. group 3 included 62 cycles, with mean age of 30±3.99, mean BMI 27.12±4.71, mean AMH 2.73±1.02, mean vitamin d level 97.08±28.82 nmol/l.

The outcome of IVF cycles in group 1, 1582 oocyte collected with mean of 14.65±7.65, 1281 mature oocyte with mean of 11.86±6.63, 979 fertilized oocyte with mean of 9.06±5.59, 956 cleaved oocyte with mean of 8.85±5.49, 379 blastocyst with mean of 3.51±2.51. In group 2, 1439 oocyte collected with mean of 13.83±7.20, 1123 mature oocytes with mean of 10.80±5.85, 871 fertilized oocytes with mean of 8.38±5.02, 832 cleaved oocytes with mean of 8.00±5.02, 328 blastocyst with mean of 3.16±2.85. In group 3:801 oocyte collected with mean of 25.43±5.85, 636 mature with mean of 10.26±5.17, 475 fertilized oocytes with mean of 7.66±4.63, 467 cleaved oocytes with mean of 7.53±4.56, 195 blastocyst with mean 3.15±2.93.

From other point of view, we evaluated the rate of oocyte and embryo performance in the three groups and compared the results.

Group 1 we found maturation rate of 81.41%, fertilization rate of 75.90%, cleavage rate per fertilized oocyte of 98.01%, cleavage rate per mature oocyte was 74.43%, blastocyst formation per cleaved oocyte rate of 60.60%. Blastocyst formation per fertilized oocyte of 59.36%, blastocyst formation per mature oocyte of 44.80%. In group 2, maturation rate was 79.43%, fertilization rate of 78.04%, cleavage rate per fertilized oocyte of 94.59%, cleavage rate per mature oocyte of 74.61%, blastocyst formation rate per cleaved oocyte was 61.02%, per fertilized oocyte 57.47%, per mature oocyte was 44.76%. In group 3, maturation rate of 78.71%, fertilization rate was 72.63%, cleavage rate per fertilized oocyte was 98.72%, cleavage rate per mature oocyte 71.55%, blastocyst rate per cleaved oocytes was 58.41%, blastocyst per fertilized oocyte was 57.66%, blastocyst per mature oocyte obtained was 42.38%.

Table 1: Demographic data of the patients.

Parameters	All	G1	G2	G3	P value
Number	273	108 (39.56%)	103 (37.73%)	62 (22.71%)	0.038
Age (years)	30±3.99	30±3.64	30±4.32	30±3.99	0.127
BMI	27.67±5.48	28.55±5.48	26.72±4.49	27.12±4.71	0.187
AMH	2.85±1.04	3.06±1.04	2.74±0.97	2.73±1.02	0.753
Vitamin D	57.9±27.9	35.85±9.03	60.73±6.24	97.08±28.82	0.004

Table 2: Cycles outcome.

Parameters	G1	G2	G3	P value
Oocyte	1582-14.65±7.62	1439-13.83±7.20	801-25.43±5.85	0.746
Mature	1281-11.86±6.63	1123-10.80±5.85	636-10.26±5.17	0.649
Fertilized	979-9.06±5.59	871-8.38±5.02	475-7.66±4.63	0.602
Cleaved	956-8.85±5.49	832-8.00±5.02	463-7.53±4056	0.601
Blastocyst	379-3.51±2.51	328-3.16±2.85	195-3.15±2.93	0.662

Table 3: Cycles outcome rate.

Parameters	G1 (%)	G2 (%)	G3 (%)	P value
Maturation rate	81.41	79.43	78.71	0.533
Fertilization rate	75.90	78.04	72.63	0.173
Cleavage rate	74.43	74.61	71.55	0.564
Blastocyst/mature oocyte	44.80	44.76	42.38	0.771
Blastocyst/fertilized oocyte	59.36	57.47	57.66	0.850
Blastocyst/cleaved oocyte	60.60	61.02	58.41	0.817

DISCUSSION

It is well known that vitamin D. Affect the fertility in several ways mainly on pregnancy outcome. But the direct effect of vitamin D was suggested through its influence on oocyte, sperm performance and embryo quality, this is beside its impact on endometrium and implantation. In our research when we choose patients almost in the same age group, same ovarian reserve, no sever sperm factors and no chromosomal factors. We found no significant difference in the laboratory outcome which is an indication for the performance of the gametes without other factors interfering. in this study we found that the prevalence of low vitamin D level was high in patients seeking IVF treatment and we can see that the number of cycles in group 1 and 2 were significantly higher than in group 3 which is for patient with normal level of vitamin D, this agrees of studies with multiple studies done all over the

world Paffoni et al, and Farzadi et al.[23,24] So, the correlation between vitamin D concentration and IVF outcome was studied extensively in a lot of researches but controversial results have been found about the mechanism of vitamin D effect weather through effect on the gamete and embryos performance or on the endometrium but in all there is an agreement that it affect the implantation and clinical pregnancy outcome as in studies by Justin et al 2019 and Jennifer et al in 2022.[25,26] In our study we could not find any significant relation between vitamin D level and the laboratory outcome which agrees with a study done by Ha et al who concluded that serum vitamin D did not affect the IVF laboratory or clinical outcome and another study done by Jason et al who found that the vitamin D level don't affect the IVF out come in case of transfer euploid blastocyst.[27,28]

But in a study done Wals et al found that the vitamin level affects the blastocyst formation rate which is against what we concluded in our study.[29] On the other hand, we can say the lower pregnancy rate obtained in patients with vitamin D deficiency was due to its effect on the endometrium which was approved by a study done by a study done by Rudick et al in 2014 who found strong relation between vitamin D level of recipients of donor oocytes and the results of IVF.[30] While in other study done by Fabris et al in 2014 concluded that no relation between vitamin D levels and IVF outcome in donor oocytes recipients.[31]

Limitations

Limitations of our study included the design of the study and the sample size of the research; we only evaluated the laboratory out come without the ploidy status of the embryos. We did not evaluate the clinical outcome, so larger prospective studies are needed for further evaluation.

CONCLUSION

Despite the proved role of vitamin D in reproduction yet we failed to find any effect of vitamin D deficiency on IVF laboratory outcome. The effect of vitamin D outcome could be mediated through affection of endometrial receptivity and implantation specially in normal responding patients aged 35 years older or less. We suggested larger prospective study in these group of patients to prove or disapprove our conclusion.

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