ROLE OF FOLLICULAR AND SERUM VITAMIN D IN THE PREDICTION OF EMBRYO QUALITY, CLINICAL PREGNANCY, AND ONGOING PREGNANCY AFTER 12 WEEKS GESTATION IN ICSI PATIENTS

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Abstract

Human and animal studies suggest that vitamin D plays an important role in Human Reproduction. This work aimed to study the role of Follicular and serum Vitamin D in the prediction of Embryo quality, clinical and ongoing pregnancy after 12 weeks in ICSI patients

Methods The study population included 88 women who went into their cycles of intra-cytoplasmic sperm injection (ICSI) treatment. The demographic parameters of the patients were evaluated. Follicular and serum 25(OH) D concentrations were measured. This work evaluated the levels of follicular and serum vitamin D and studied its correlation with Embryo quality, clinical pregnancy, and ongoing pregnancy after 12 weeks gestation in ICSI patients.

Results The FF 25(OH) D ranged from 11.1 to 25.1 (ng/ml) with a mean FF 25(OH) D ± SD of 18.7±4.6. The Serum 25(OH) D ranged from 20.1-29.8 with a mean Serum 25(OH) D ± SD of 25.7±3.7. The positive pregnancy test was found in 32 patients representing a 36.4% success rate. Good Quality embryos were obtained on the second day of the culture in 40 patients which represent 45.5%. Ongoing pregnancy at 12 weeks was obtained in 28 patients which represents 31.8%. There was a highly statistically significant difference between those who got clinical pregnancy and those who did not get clinical pregnancy regarding weight and BMI, p-value < 0.001. There was a statistically significant difference between those who got clinical pregnancy and those who did not get clinical pregnancy regarding Age and height, p-value =0.002 and 0.007 respectively. The optimal cutoff point for FF 25(OH)D for prediction of clinical pregnancy was ≤ 14.7 ng/ml, the area under the curve was 0.815, the 95% CI was 0.718-0.890, P value <0.001, Sensitivity 87.5, Specificity 92.86, positive predictive value (PPV) 87.5, negative predictive value (NPV) 92.9 and accuracy was 90.91. While the curve analysis for Serum 25(OH) D showed the same figures but with the optimal cutoff point for prediction of clinical pregnancy was ≤ 23.2 ng/ml. The optimal cutoff point for FF 25(OH)D for prediction of Embryo Quality was ≤ 19.5 ng/ml, the area under the curve was 0.773, the 95% CI was 0.671-0.855, P value <0.001, Sensitivity 80, Specificity 91,67, positive predictive value (PPV) 88.9, negative predictive value (NPV) 84.6 and accuracy was 86.4. While The optimal cutoff point for Serum 25(OH)D for prediction of Embryo Quality was ≤ 23.2 ng/ml, the area under the curve was 0.760, the 95% CI was 0.658-0.845, P value <0.001, Sensitivity 70, Specificity 91,67, positive predictive value (PPV) 87.5, negative predictive value (NPV) 78.6 and accuracy was 81.8. The optimal cutoff point for FF 25(OH)D for prediction of ongoing pregnancy at 12 weeks was ≤ 14.7 ng/ml, the area under the curve was 0.755, the 95% CI was 0.651-0.840, P value <0.001, Sensitivity 85.71, Specificity 86.67, positive predictive value (PPV) 75, negative predictive value (NPV) 92.9 and accuracy was 86.4. While the curve analysis for Serum 25(OH) D showed the same figures but with the optimal cutoff point for prediction of clinical pregnancy was ≤ 23.2 ng/ml.

Conclusions Follicular Fluid 25(OH) D and Serum 25(OH) D are good predictors of Clinical pregnancy, Embryo quality, and ongoing pregnancy at 12 weeks gestation.

Keywords: vitamin D, follicular fluid and Serum vitamin D, ICSI outcome.

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Introduction

The well-known actions of vitamin D include calcium and phosphorus homeostasis in addition to the support of bone Mineralization, while its deficiency leads to an increased risk of muscle weakness, osteoporosis, and bone fractures (1). Recent years have witnessed a shift in focus to the non-skeletal benefits of vitamin D; in this latter context, an accruing body of literature attests to the relevance of vitamin D to reproductive physiology (2). Vitamin D receptors (VDR) have been identified in the female reproductive tissues including human ovarian, endometrial, and fallopian tube epithelial cells, placenta, and decidual cells (3). Vitamin D via its receptor can modulate ovarian steroidogenesis (4). Additionally, data from clinical reports suggest that lower vitamin D level is associated with gestational diabetes and preeclampsia. Expression of VDR is also increased during pregnancy (4, 5). Vitamin D can contribute to the restoration of the menstrual cycle and endometrial proliferation (7, 8), promote the development of follicles (9), ameliorate primary dysmenorrhea (10), and reduce the occurrence of uterine fibroids (11). All these mentioned effects of vitamin D led some authors to consider vitamin D as a steroid hormone (12).

However, the relationship of vitamin D levels with ICSI outcomes remains controversial. Although Polyzos et al., 2014 reported that women with deficient serum vitamin D had a lower clinical pregnancy rate compared to women with normal levels of vitamin D (13), other studies led to opposite conclusions (14-19). Because of these conflicting reports, and given that vitamin D deficiency is not correlated with ovarian stimulation characteristics or with markers of embryo quality (16, 20). It has been postulated that vitamin D deficiency may negatively affect pregnancy rates in IVF with an effect mediated through the endometrium (13, 16, and 17). In both the epithelial and stromal cells of the endometrium, vitamin D induces and up-regulates the transcription of HOXA 10, a gene essential for implantation with increased expression during the window of implantation (20, 21). Conversely, when a transfer of synchronous euploid blastocyst is performed, the vitamin D status does not impact the outcomes (15). Most of the published studies on vitamin D and assisted reproduction technologies (ART) have reported outcomes related to clinical pregnancy rates or live birth rates. However, these studies were not designed to evaluate the direct relationship of local-intra-follicular vitamin D levels with the ability of the individual oocyte to undergo fertilization and subsequent embryo development (14, 15, 16, 19, 23, and 24).

This work aimed to evaluate the role of Follicular and serum Vitamin D in the prediction of Embryo quality, clinical pregnancy, and ongoing pregnancy after 12 weeks gestation in ICSI patients.

Patients and Methods

Patients' selection

The study protocol was approved by the Ethics Committee of the Department of Obstetrics and Gynecology, Faculty of Medicine, and Minia University. This is a prospective observational study that was conducted in the Department of Obstetrics and Gynecology, Faculty of Medicine, Minia University during the period from July 2019 to December 2020. The study recruited patients referred for assisted reproduction treatment (ART) cycles to two of Minia's private centers for assisted reproduction under the supervision of the principal investigator according to study protocol and written informed consent was obtained from each patient after explanation of the procedure. The study population included 88 women who went into their cycles of intra-cytoplasmic sperm injection (ICSI) treatment. All included patients fulfilled the following inclusion criteria: their ages between 21 and 38 years, Their body mass indexes (BMI between 18 and 29 kg/m2), day 3 serum FSH levels <12 IU/L, their menstrual cycles are regular between 24 and 35 days, no previous history of ovarian surgery and all are well unexplained infertility. While exclusion criteria included those with moderate to severe male factor infertility, Poor responders. None of the patients received hormonal therapy in the previous 3 months and none of them received vitamin D supplementation before controlled ovarian hyper-stimulation (COH).

Induction Protocol and follow up

All patients received standard controlled ovarian hyperstimulation (COH) with recombinant FSH (r-FSH) under pituitary suppression with a GnRH agonist.

Oocyte retrieval was performed using trans-vaginal ultrasound 34 to 36 h after hCG injection. Intracytoplasmic sperm injection (ICSI) was performed using standard procedures and the embryos were transferred 2, 3, or 5 days later. The luteal phase was supported with 40 mg progesterone administration by daily injection. A pregnancy test was carried out on Day 14 after embryo transfer. Two weeks later, a transvaginal ultrasound was performed to confirm pregnancy. Study endpoints: Fertilization rate (FR) was calculated as the number of fertilized oocytes relative to the number of retrieved oocytes. Good quality embryos were defined as those at the 4 to 6-cell stage on Day 2. The good-quality embryos meeting these criteria were either transferred (ET) to the recipients or frozen. Biochemical pregnancy was defined by the presence of β-HCG >50 mIU/mL without ultrasound evidence of a gestational sac. Clinical pregnancy was defined by the presence of a gestational sac.

25(OH) D Measurement

25-Hydroxyvitamin D is the primary circulating form of vitamin D and remains stable throughout the menstrual cycle (25). Due to its stability, serum 25(OH) D concentration is viewed as the best indicator of vitamin D status (26). Heparinized serum samples and follicular fluid samples from all patients were obtained on the day of oocyte retrieval and kept frozen at -80 °C until the measurement was performed. 25(OH) D level was measured using the chemiluminescent immunoassay (CLIALIAISON®) 25 OH Vitamin D TOTAL Assay (REF 310600) DiaSorin Inc., Stillwater, MN, USA. Vitamin D deficiency was defined by the Institute of Medicine (IOM) and the Endocrine Society Clinical Practice Guidelines (27, 28) as serum 25(OH) D level < 20 ng/ml. The follicular fluid vitamin D norm remains unknown.

Follicular fluid (FF) collection

FF samples were collected according to the strict procedure described by Ciepiela P et al., 2015 (29). To collect clear follicular fluid and to avoid multiple vaginal punctures and the numerous flushing of the needle with culture medium after every follicular puncture, as well as to minimize the risk of vaginal bleeding, we decided to include in the study FF only from the first nearest (available) aspirated (lead) follicle of each ovary. Consequently, vitamin D levels were representative for the lead follicle only, and not for the entire cohort of follicles from any given patient. Each ovarian follicle was aspirated independently and collected in a separate test tube to identify the matched single cumulus–oocyte–complex (COC). This approach was chosen to avoid cross-contamination from the flush medium or the FF of other follicles. Test tubes with more than one COC were excluded from the study. In each case collected, FF was checked afterward for red blood cells. Fluids with red blood cells were disqualified. FF samples with matched mature metaphase II (MII) oocytes were centrifuged at 10,000×g for 10 min, and the supernatants we reallocated and stored at –C80°.

Intracytoplasmic sperm injection (ICSI)

This study included only women undergoing ICSI to allow a precise cumulus–oocyte–complex evaluation, especially about its maturity/degradation before fertilization. Furthermore, during conventional IVF insemination, there are significant events that take place, such as sperm penetration through layers of supporting granulosa cells, sperm membrane breaching, and fusion; thus, IVF requires competent oocytes and capable spermatozoa. All these events are circumvented by the ICSI procedure. While ICSI may affect the integrity of an oocyte, the literature supports the concept that oocyte ICSI degeneration is operator-independent (30).

Embryo assessment

Embryos underwent regular embryo assessment as described by the 2011 Istanbul consensus (30). A top-quality embryo (TQE) on the second day of the culture was defined as an embryo with four symmetrical, non-fragmented blastomeres (31).

Outcome measures

This study aimed to evaluate the role of follicular and serum Vitamin D in the prediction of Embryo quality, clinical pregnancy, and ongoing pregnancy after 12 weeks gestation in ICSI patients. To examine the development competence of oocytes, the following parameters were taken into consideration:

1- Ability of the oocyte to form a good quality embryo after ICSI

2- Positive hCG rate after embryo transfer (ET), measured 14 days after embryo transfer.

3- Clinical pregnancy rate after ET is defined as the presence of an intrauterine sac with an embryonic pole demonstrating cardiac activity at 7 weeks of gestation.

4- Continuing pregnancy up to 12 weeks of gestation.

Statistical method

The analysis of the data was carried out using the IBM SPSS version 25 statistical package software. The normality of the data was tested using the Kolmogorov-Smirnov test and Shapiro-Wilk test. Data were expressed as mean ± SD and minimum and maximum range for parametric quantitative data and by median (IQR) for non-parametric quantitative data, in addition to both number and percentage for qualitative data. Analyses were done between the two groups for parametric quantitative data using the Independent Samples T-test and non-parametric quantitative data using the Mann-Whitney test. In contrast, the Chi-square test was used to compare categorical variables. Correlation between variables was done using Pearson's correlation for continuous variables and Spearman's correlation for ordinal variables. Receiver Operating Characteristic (ROC) curve was used to determine the AUC, sensitivity, specificity, PPV, NPV, and accuracy of the Vit. D-level pregnancy and embryo quality. A P-value less than 0.05 was considered statistically significant.

Results

Patients` demographic data

Eighty-eight patients were included in the study. Their age ranged from 21 to 38 with a mean age ± SD of 28.5±5.3. Their weight ranged from 45 to 90.9 KG with a mean weight ± SD of 63.5± 12.5. Their height ranged from 1.5-1.8 M with a mean height ± SD of 1.6±0.1. Their Body Mass Index (BMI) ranged from 18 to 29.2 with a mean BMI ± SD of 23.6±3.4. The interquartile range of their parity was 0 to 2, with a mean of 1. Twenty-four of the patients were having primary infertility and sixty-four were having secondary infertility. The interquartile range of their duration of infertility was 2 to 4, with a mean of 3 (Table 1).

Table 1: Demographic data

		Descriptive statistics N=88
Age	*Range* *Mean ± SD*	(21-38) 28.5±5.3
Weight (KG)	*Range* *Mean ± SD*	(45-90.9) 63.5±12.5
Height (M)	*Range* *Mean ± SD*	(1.5-1.8) 1.6±0.1
BMI	*Range* *Mean ± SD*	(18-29.2) 23.6±3.4
Parity	*Median* *IQR*	1 (0-2)
Duration of infertility (Ys)	*Median* *IQR*	3 (2-4)

Intra-cytoplasmic Sperm Injection Data

Day 3 Serum FSH (IU/L) ranged from 5.1 to 11.6 with a mean level ± SD of 9.2±2.2. Forty (45.5%) of the embryos were transferred on day two, 32(36.4%) of the embryos were transferred on day three, 16 (18.2%) of the embryos were transferred on day five. The interquartile range of Number of retrieved Oocytes was 4 to 5, with a mean of 4. The interquartile range of Number of fertilized Oocytes was 2 to 5, with a mean of 3. The fertilization rate ranged from 50 to 100 with a mean level ± SD of 70.7±14.8 . The interquartile range of Number of transferred Embryos was 1 to 1, with a mean of 1. The interquartile range of Number of Frozen Embryos was 1 to 4, with a mean of 1 (Table 2). Thirty two (36.4%) have positive pregnancy test on day 14 (β-HCG >50 mIU/mL) , 56 (63.6%) have negative pregnancy test on day 14 (β-HCG >50 mIU/mL) (Table 2).

Table 2: ICSI data

		Descriptive statistics N=88
Day 3 Serum FSH (IU/L)	*Range* *Mean ± SD*	(5.1-11.6) 9.2±2.2
Day of Embryo Transfer	*Median* *IQR*	3 (2-3)
	2 *3* *5*	40(45.5%) 32(36.4%) 16(18.2%)
Number of Retrieved Oocytes	*Median* *IQR*	4 (4-5)
Number of Fertilized Oocytes	*Median* *IQR*	3 (2-5)
Fertilization rate	*Range* *Mean ± SD*	(50-100) 70.7±14.8
Number of transferred Embryos	*Median* *IQR*	1 (1-1)
	1 *2*	68(77.3%) 20(22.7%)
Number of Frozen Embryos	*Median* *IQR*	1 (1-4)
BHCG on day 14	*Median* *IQR*	5 (2-96)
	-Ve *+Ve*	56(63.6%) 32(36.4%)

Serum 25(OH) D and Follicular fluid level

The FF 25(OH)D ranged from 11.1 to 25.1 (ng/ml) with a mean FF 25(OH)D ± SD of 18.7±4.6. The

Serum 25(OH) D ranged from 20.1-29.8 with a mean Serum 25(OH)D ± SD of 25.7±3.7. (Table 3 and Figure 1).

Table 3: Follicular fluid and serum Vitamin D level.

		Descriptive statistics N=88
FF 25(OH)D	*Range* *Mean ± SD*	(11.1-25.1) 18.7±4.6
serum 25(OH)D	*Range* *Mean ± SD*	(20.1-29.8) 25.7±3.7

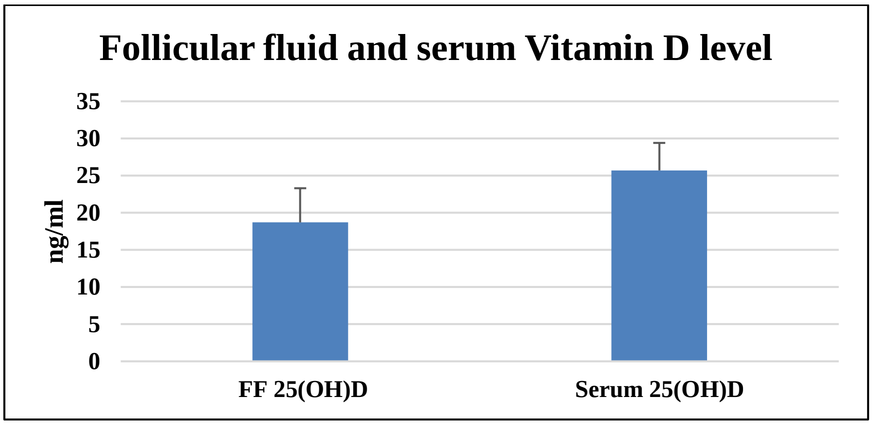

Figure 1: Follicular fluid and serum Vitamin D level

Pregnancy outcome

The positive pregnancy test was found in 32 patients representing a 36.4% success rate. Good Quality embryos were obtained on the second day of the culture in 40 patients which represent 45.5%. Ongoing pregnancy at 12 weeks was obtained in 28 patients which represents 31.8% (Table 4 and Figure 2).

Table 4: Pregnancy outcome

		Descriptive statistics N=88
Clinical Pregnancy	*Negative preg. test* *Positive preg .test*	56(63.6%) 32(36.4%)
Embryo Quality ((TQE) on the second day of the culture)	*Bad quality Emb.* *Good Quality Emb.*	48(54.5%) 40(45.5%)
Ongoing Pregnancy At 12 weeks	*Failed preg.* *Ongoing preg.*	60(68.2%) 28(31.8%)

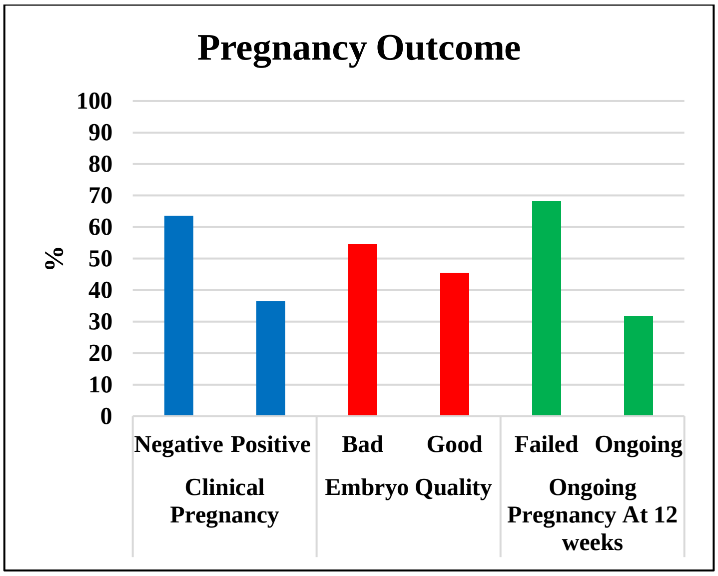

Figure 2: Pregnancy outcome with Clinical pregnancy, Embryo quality and ongoing pregnancy at 12 weeks

Vitamin D for predication of clinical pregnancy

The optimal cutoff point for FF 25(OH)D for prediction of clinical pregnancy was ≤ 14.7 ng/ml, the area under the curve was 0.815, the 95% CI was 0.718-0.890, P value <0.001, Sensitivity 87.5, Specificity 92.86, positive predictive value (PPV) 87.5, negative predictive value (NPV) 92.9 and accuracy was 90.91. While the curve analysis for Serum 25(OH) D showed the same figures but with the optimal cutoff point for prediction of clinical pregnancy was ≤ 23.2 ng/ml (table 5 and Figures 3&4).

Table 5: ROC curve analysis of vitamin D for predication of clinical pregnancy

	FF 25(OH)D	Serum 25(OH)D
Optimal cutoff point	**≤ 14.7 ng/ml**	**≤ 23.2 ng/ml**
AUC	**0.815**	**0.815**
95% CI	**0.718-0.890**	**0.718-0.890**
P value	***<0.001****	***<0.001****
Sensitivity	**87.5**	**87.5**
Specificity	**92.86**	**92.86**
PPV	**87.5**	**87.5**
NPV	**92.9**	**92.9**
Accuracy	**90.91**	**90.91**

- ROC curve analysis
- AUC: Area under Curve
- CI: Confidence Interval
- PPV: Positive Predictive Value
- NPV: Negative Predictive Value
- *: Significant level at P value < 0.05

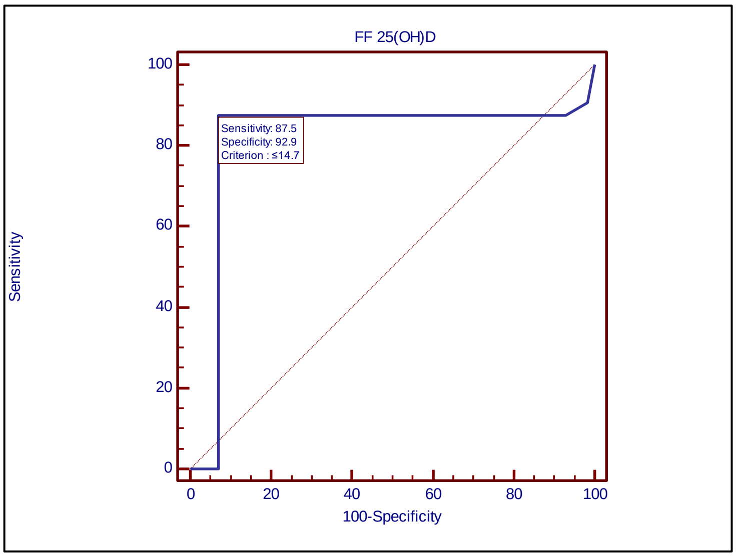

Figure 3: ROC curve analysis of FF 25(OH) D for predication of clinical pregnancy

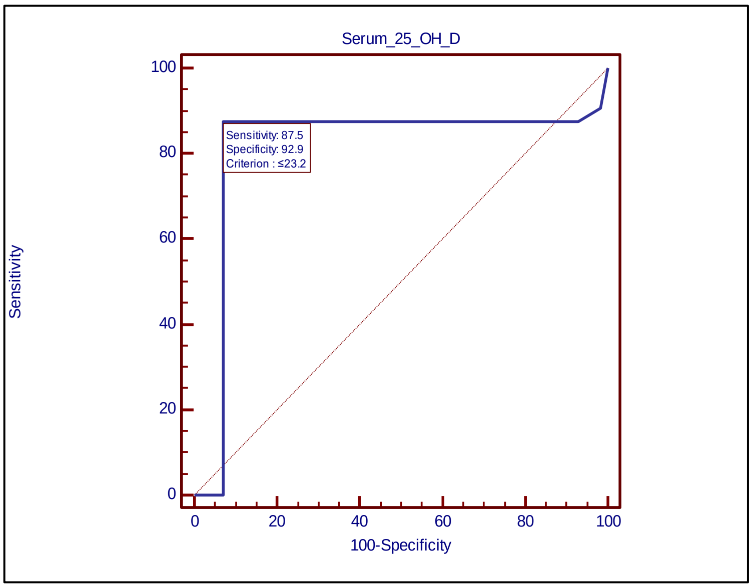

Figure 4: ROC curve analysis of Serum 25(OH) D for predication of clinical pregnancy

Vitamin D for predication of embryo quality

The optimal cutoff point for FF 25(OH)D for prediction of Embryo Quality was ≤ 19.5 ng/ml, the area under the curve was 0.773, the 95% CI was 0.671-0.855, P value <0.001, Sensitivity 80, Specificity 91,67, positive predictive value (PPV) 88.9, negative predictive value (NPV) 84.6 and accuracy was 86.4. While The optimal cutoff point for Serum 25(OH)D for prediction of Embryo Quality was ≤ 23.2 ng/ml, the area under the curve was 0.760, the 95% CI was 0.658-0.845, P value <0.001, Sensitivity 70, Specificity 91,67, positive predictive value (PPV) 87.5, negative predictive value (NPV) 78.6 and accuracy was 81.8. (Table 6 and figures 5&6).

Table 6: ROC curve analysis of vitamin D for predication of embryo quality

	FF 25(OH)D	Serum 25(OH)D
Optimal cutoff point	≤ 19.5	≤ 23.2
AUC	0.773	0.760
95% CI	0.671-0.855	0.658-0.845
P value	***<0.001****	***<0.001****
Sensitivity	80	70
Specificity	91.67	91.67
PPV	88.9	87.5
NPV	84.6	78.6
Accuracy	86.4	81.8

- ROC curve analysis
- AUC: Area under Curve
- CI: Confidence Interval
- PPV: Positive Predictive Value
- NPV: Negative Predictive Value
- *: Significant level at P value < 0.05

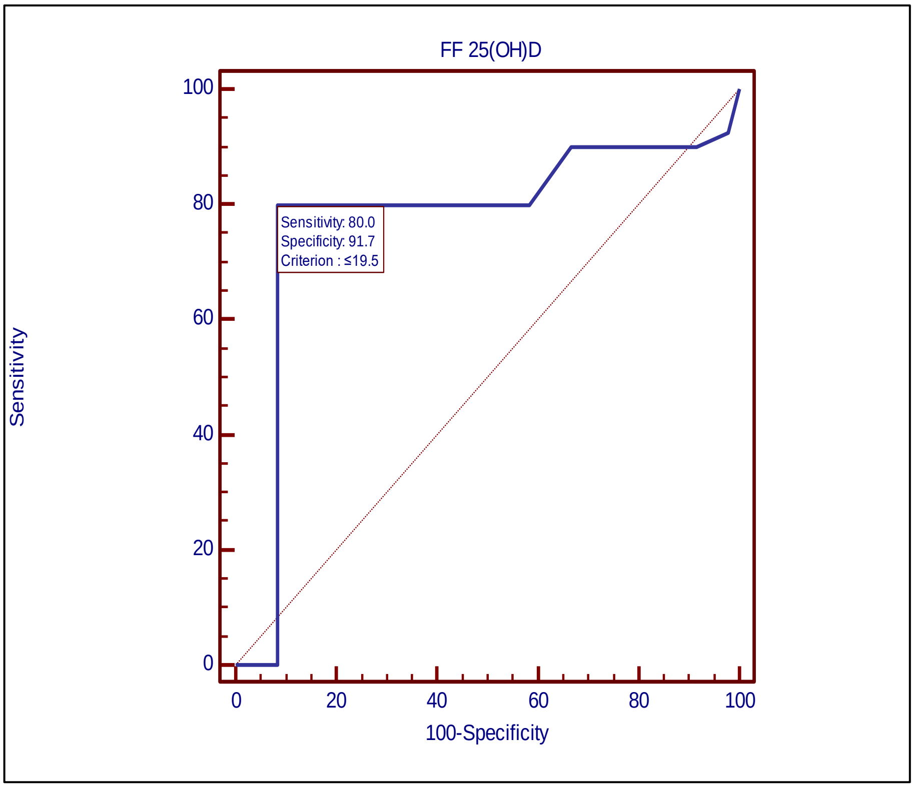

Figure 5: ROC curve analysis of FF 25(OH)D for predication of Embryo Quality

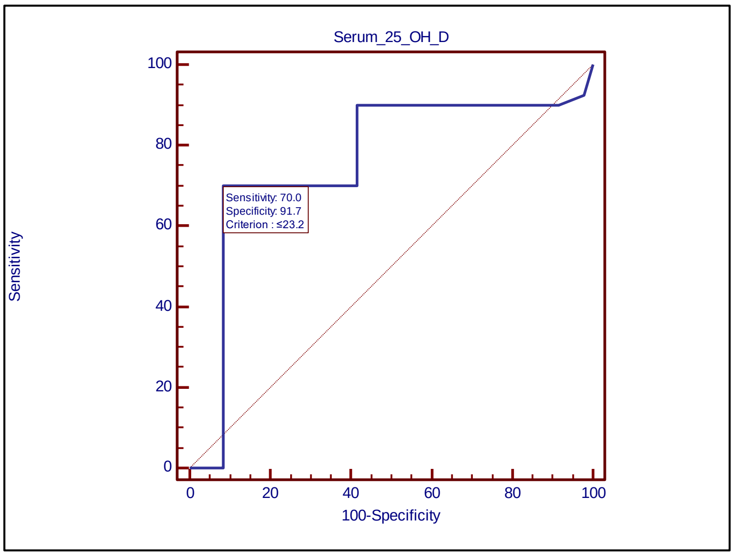

Figure 6: ROC curve analysis of FF Serum (OH) D for predication of Embryo Quality

Vitamin D for predication of ongoing pregnancy at 12 weeks

The optimal cutoff point for FF 25(OH)D for prediction of ongoing pregnancy at 12 weeks was ≤ 14.7 ng/ml, the area under the curve was 0.755, the 95% CI was 0.651-0.840, P value <0.001, Sensitivity 85.71, Specificity 86.67, positive predictive value (PPV) 75, negative predictive value (NPV) 92.9 and accuracy was 86.4. While the curve analysis for Serum 25(OH) D showed the same figures but with the optimal cutoff point for prediction of clinical pregnancy was ≤ 23.2 ng/ml. (Table 7 and Figures 7&8).

Table 7: ROC curve analysis of vitamin D for predication of ongoing pregnancy at 12 weeks

	FF 25(OH)D	Serum 25(OH)D
Optimal cutoff point	≤ 14.7	<23.2
AUC	0.755	0.769
95% CI	0.651-0.840	0.667-0.852
P value	***<0.001****	***<0.001****
Sensitivity	85.71	85.71
Specificity	86.67	86.67
PPV	75	75
NPV	92.9	92.9
Accuracy	86.4	86.4

- ROC curve analysis
- AUC: Area Under Curve
- CI: Confidence Interval
- PPV: Positive Predictive Value
- NPV: Negative Predictive Value
- *: Significant level at P value < 0.05

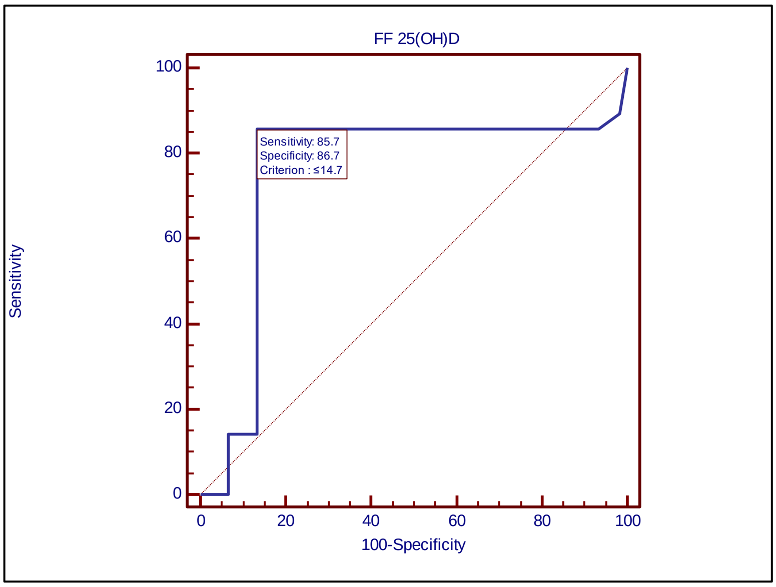

Figure 7: ROC curve analysis of FF 25(OH)D for predication of ongoing pregnancy at 12 weeks

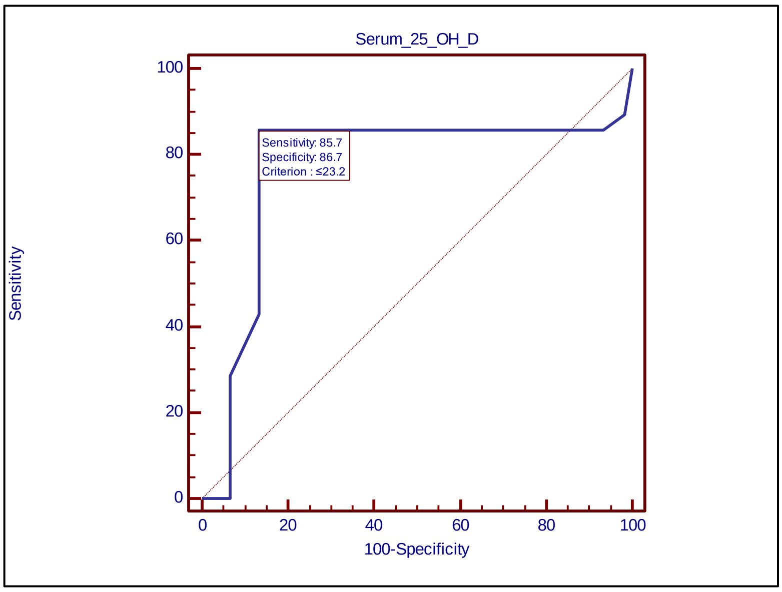

Figure 8: ROC curve analysis of Serum 25(OH)D for predication of ongoing pregnancy at 12 weeks

Discussion

The recent advances in Assisted Reproductive Technologies (ART) and bioassays made it possible to study the relationship of both Follicular fluid (FF) and serum levels of vitamin D with the different steps of ICSI including oocyte fertilization, embryo development, and pregnancy outcome. Despite several scientific research which tried to study these relations (25, 31, 32, and 33), the relationship between the level of vitamin D (FF and serum) and ICSI outcome(s) remains controversial. It seems that the level of vitamin D has a complex relationship with ICSI outcome. The previous systemic reviews and meta-analyses of serum vitamin D and ICSI outcomes do not support the idea of routinely screening 25(OH)D serum status to predict the clinical pregnancy rate, nor supplementing vitamin D in couples undergoing ART (25, 31, 32).

In this current study, there was a highly statistically significant difference between those who got clinical pregnancy and those who did not get clinical pregnancy regarding weight and BMI, with p value < 0.001. These results are in agreement with Rehman R. et al 2013 (34) who concluded that a BMI cut-off value of above 26 kg/m2 in their study population is associated with a negative impact on pregnancy outcome. However, our results are not in agreement with Adel El Sayed, 2007 (35) who concluded that being overweight and obese did not affect the ICSI outcome. This discrepancy in the result among different studies could be attributed to the differences in the studied populations.

The optimal cutoff point for FF 25(OH)D for prediction of clinical pregnancy was ≤ 14.7 ng/ml, the area under the curve was 0.815, the 95% CI was 0.718-0.890, P value <0.001, Sensitivity 87.5, Specificity 92.86, positive predictive value (PPV) 87.5, negative predictive value (NPV) 92.9 and accuracy was 90.91. While the curve analysis for Serum 25(OH) D showed the same figures but with the optimal cutoff point for prediction of clinical pregnancy was ≤ 23.2 ng/ml. these findings are in general agreement with those of Aleyasin et al, 2011(19) who studied a comparable sample of infertile women and found a significant correlation between the levels of vitamin D in follicular fluid and serum. The overall rates of chemical, clinical, and ongoing pregnancy were 35.5 % (n=29), 29.3% (n-24), and 23.2 % (n=19) respectively. The median values of FF Vitamin D and Serum Vitamin D in their study were generally less than those of this current study. The explanation of this difference is attributed to the fact that the level of Vitamin D both in FF and serum is a reflection of body stores of Vitamin D and that most of their patients were deficient in vitamin D as they stated. This current study cut off limit for both FF and serum Vitamin D (≤ 14.7 ng/ml and ≤ 23.2 ng/ml respectively), These findings are not in agreement with Hasan HA et al, 2023 (36) who recommended a serum 25(OH) D cut off level of ≥ 50 nmol/ for women to undergoing IVF therapy. However, more studies with larger sample sizes are needed to verify which of these results are to be used in the future.

The optimal cutoff point for FF 25(OH)D for prediction of Embryo Quality was ≤ 19.5 ng/ml, the area under the curve was 0.773, the 95% CI was 0.671-0.855, P value <0.001, Sensitivity 80, Specificity 91,67, positive predictive value (PPV) 88.9, negative predictive value (NPV) 84.6 and accuracy was 86.4. While The optimal cutoff point for Serum 25(OH)D for prediction of Embryo Quality was ≤ 23.2 ng/ml, the area under the curve was 0.760, the 95% CI was 0.658-0.845, P value <0.001, Sensitivity 70, Specificity 91,67, positive predictive value (PPV) 87.5, negative predictive value (NPV) 78.6 and accuracy was 81.8. These findings are in general agreement with Ciepiela et al, 2018(37).

To focus on the evaluation of oocyte competence, we excluded couples with moderate and severe male factors and therefore minimized the role of spermatozoa as a factor affecting embryo development. In contrast, in some previous studies, the male factor was present in 58–65% of couples (14, 24), while in other studies (16, 19, and 24), the indications for IVF/ ICSI were not mentioned. Additionally, to test only mature oocytes, we evaluated only patients undergoing ICSI.

The optimal cutoff point for FF 25(OH)D for prediction of ongoing pregnancy at 12 weeks was ≤ 14.7 ng/ml, the area under the curve was 0.755, the 95% CI was 0.651-0.840, P value <0.001, Sensitivity 85.71, Specificity 86.67, positive predictive value (PPV) 75, negative predictive value (NPV) 92.9 and accuracy was 86.4. While the curve analysis for Serum 25(OH) D showed the same figures but with the optimal cutoff point for prediction of clinical pregnancy was ≤ 23.2 ng/ml. these findings are not in agreement with Hassan SS et al, 2022 (38) who concluded that there was no statistically significant relation between vitamin level and pregnancy outcome in women with unexplained infertility undergoing induction of ovulation. There also concluded that vitamin D level has limited predictive value in women with unexplained infertility undergoing induction of ovulation and the best cutoff value was < 32.5 ng/ml.

Conclusions

Follicular Fluid 25(OH) D and Serum 25(OH) D are good predictors of Clinical pregnancy, Embryo quality, and ongoing pregnancy at 12 weeks gestation.

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