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MEASUREMENT OF SERUM PROGESTERONE LEVELS ON THE DAY OF EMBRYO TRANSFER IS A USEFUL TOOL IN PREDICTION OF SUCCESSFUL PREGNANCY

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Abstract: Progesterone plays a key role in the establishment of endometrial receptivity and embryo implantation. However, knowledge on the prognostic significance of serum progesterone on the day of embryo transfer in natural cycle is still scarce.

A single center prospective study of single frozen embryo transfer (SFET) was performed during the period October 2015 – November 2016 with 252 women undergoing ICSI cycles. Progesterone concentrations were measured on the day of SFET and pregnancy test (bhCG) was done 14 days later. Receiver Operator Characteristic curve (ROC) was used to evaluate the predictive value of serum progesterone for discriminating women with successful implantation and to delineate the optimal cut-off value.

Successful implantation registered by bhCG test occurred in 44.8% of the patients. There were no significant differences in age, FSH, BMI and quality of the transferred embryos between pregnant and non-pregnant women. Mean serum progesterone levels were significantly lower in women with negative bhCG-test compared with those with positive pregnancy test (20.08 ng/ml ± 9.44 vs. 27.9 ng/ml ± 10.52 respectively ($p=0.034$)). At cut-off value of 14.40 ng/ml, progesterone had 88% sensitivity and 75% specificity. The area under the ROC curve for progesterone was 0.67 (95% Confidence Interval, 0.52-0.89).

In conclusion, these findings indicate that serum progesterone, measured on the day of embryo transfer could be used for prediction of successful implantation.

INTRODUCTION

Progesterone (P4) is an essential factor that is required for endometrial gland formation and contributes to the success of embryo implantation (Day et al., 2009). The midluteal serum P4 concentration is usually between 10 and 20 ng/mL (Sallam et al., 1999).

Most of the studies have focused on the identification and application of the optimal regime for luteal support after embryo transfer (ET) (Hubayter and Muasher, 2008; Glujovsky et al., 2010; van der Linden et al., 2011). Although most of them have achieved an increase in live birth rate with P4 luteal support, it is not confirmed whether P4 support could directly affect the miscarriage or biochemical pregnancy rate (Kim et al., 2014). On the other hand, relatively few studies have addressed the predictive value of serum progesterone on the day of embryo transfer (Brady et al., 2014; Kofinas et al., 2015). It was shown that high levels of P4 during the day of fresh ET lead to decreased uterine contractility and higher implantation rates (Fanchin et al., 1998). Higher levels of P4 during the pre-ovulation period and hCG administration could lead to premature luteinisation and lower implantation rates (Huang et al, 2012; Ochsenkühn et al., 2012).

The aim of this study was to assess the predictive value of the serum P4 on the day of single frozen embryo transfer on the implantation rates and to determine an appropriate cut-off value.

MATERIALS AND METHODS

Study design

This single center prospective study was carried out in the assisted reproduction unit of MHAT Nadezhda after the approval by the local institutional ethics committee. It includes only cases with single frozen embryo transfer (SFET) that were performed during the period October 2015 – November 2016 with 252 women undergoing ICSI cycles. In all cases a blastocyst stage ET was done seven days after LH surge in normal menstrual cycle. Inclusion criteria were women with age<42 yrs, basal FSH≤12 mIU/l, regular menstrual cycle and BMI: 18–28 kg/m2.

On the day of the performed embryo transfer, 5 ml venous blood was withdrawn from each participant for the determination of serum progesterone by electro-chemiluminescence immunoassay (ECLIA) on the Cobas e 411 analyser (Roche Diagnostics, Mannheim, Deutschland). The assay had a sensitivity of 0.03 ng/ml. The intra and inter-assay variation coefficients for the P4 determinations were 2.4–2.7% and 5.4–5.5%, respectively, for P4 values between 1.57 and 30.2 ng/ml. Progesterone concentrations were measured on the day of SFET and pregnancy test (bhCG) was done 14 days later.

Statistical analysis

Statistical analysis of the data was performed using SPSS v.21. Data were reported as mean ± SD. Independent-samples t-test was performed to find out whether any significant mean difference exist between the studied groups.

Receiver Operator Characteristic (ROC) curve was constructed to evaluate the level of serum P4 in distinguishing successful implantation from unsuccessful implantation. The diagnostic value of progesterone as a prognostic value in successful implantation was established by its best sensitivity, specificity and optimal diagnostic cut-off from the Receiver Operator Characteristic (ROC) curve analysis.

In all analyses, P value of less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

The baseline characteristics for the 252 patients and cycles meeting inclusion criteria are shown in Table 1. There were no significant differences between the pregnant and non-pregnant women regarding their age, BMI, FSH, the number of retrieved and fertilized oocytes, the number of growing embryos and the achieved fertilization rate. However, the serum level of P4 was significantly higher in the pregnant group, compared to the non-pregnant group (27.9 ± 10.5 ng/ml vs. 20.1 ± 9.4 ng/ml, respectively) (Table 1).

Table 1 Comparison between the study groups regarding the baseline characteristics, cycle details and the outcome measures

Characteristic	Pregnant n=113	Not pregnant n=139	P value
Age (Years)	34 ± 4.3	35 ± 4.8	0.76
BMI (kg/m2)	24.9 ± 4.3	25.2 ± 4.5	0.62
FSH (Follicular phase miU/ml)	6.5 ± 2.9	6.3 ± 3.3	0.69
Number of retrieved oocytes	24 ± 3.7	25 ± 4.2	0.82
Number of fertilized oocytes	7.2 ± 2.8	6.9 ± 3.3	0.66
Number of growing embryos	6.4 ± 1.9	6.3 ± 2.8	0.87
Fertilization rate (%)	88	85	0.54
Number of embryos transferred	1	1	-
P4 level (ng/ml)	27.9 ± 10.5	20.1 ± 9.4	**0.034***

The ROC curve analysis demonstrated the ability of serum progesterone to differentiate between successful and unsuccessful implantation of human embryo. The area under curve (AUC) for P4 was 0.67 (95% CI, 0.52-0.89) with the parameters calculated from the ROC curve (Figure 1). When using a P4 concentration of 14.4 ng/ml as a cut-off value for the prediction of implantation success, the obtained sensitivity and specificity were 88% (95% CI) and 75% (95% CI), respectively.

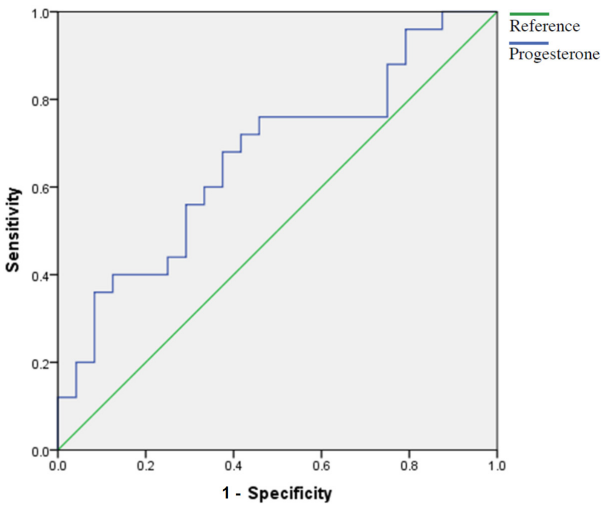

Fig. 1 Receiver operator characteristic (ROC) curve of progesterone levels as diagnostic test for successful implantation. The area under the curve for progesterone is (AUC) 0.67 (95% Confidence Interval, 0.52-0.89).

For normal implantation window to occur an estrogen and progesterone phases are of crucial importance. In the proliferative and early luteal phase progesterone and estrogen receptors are highly expressed in the endometrial tissue (Lessey et al., 1988). The increased secretion of P4 in the middle luteal phase has a down-regulating effect on estrogen receptors expression and leads to subsequent disappearance of progesterone receptors (Garcia et al., 1988).

A lot of natural cycles in assisted reproduction are associated with unsuccessful implantation even when the embryo transfer was performed with high-quality embryos. It is well known that dysfunctional endometrium also has an essential effect on pregnancy outcome (Makker and Singh, 2006). One of the possible factors that could lead to the impaired endometrial receptivity is the unbalanced progesterone and estrogen secretion (Young, 2013). Progesterone secretion has a quiescent effect on the uterus, and higher serum levels of P4 have been correlated with less uterine contractility and higher implantation rates (Fanchin et al., 1998). Patients with P4 levels above certain thresholds may have decreased myometrial activity and a relatively lower chance of displacement of the embryo from the proper position in the uterine cavity. In addition, many growth factors and membrane receptors that have an essential effect on the receptive endometrium during the "window of implantation" are modulated by P4 secretion (Halasz and Szekeres-Bartho, 2013).

There are many studies for progesterone potential to predict viable vs. non-viable pregnancy and ectopic vs. non-ectopic pregnancy (Valley et al., 1998; Florio et al., 2007; El Bishry et al., 2008; Day et al., 2009). As might be expected the threshold value of P4 is different during the day of hCG administration in comparison with the concentration during the day of ET. Previous studies have shown that progesterone levels within a relatively narrow range on the day of

HCG administration correlate with higher pregnancy rates (Xu et al., 2012; Santos-Ribeiro et al., 2014). Premature rise and the prolonged duration of the observed elevation in P4 also has undesirable effect on implantation and live-birth rate (Huang et al., 2012; Venetis et al., 2012).

Our study has evaluated the role of a serum progesterone measurement in the prediction of successful implantation in women who underwent IVF procedure and frozen embryo transfer. From the obtained results, we found that the levels of serum progesterone are significantly higher in the women with positive bhCG-test in comparison with those with negative bhCG-test. The proposed cut-off value of 14.4 ng/ml was higher as compared to the applied cut-off of 10 ng/ml to diagnose non-viable pregnancy by Dumps et al. (Dumps et al., 2002). Recently, a prospective study have shown that patients with serum P4 < 9.2 ng/ml on the day of ET had a significantly lower ongoing pregnancy rate in artificial endometrium preparation cycles (Labarta et al., 2017). Another study suggests twice greater cut-off value. In this case P4 levels above 20 ng/ml were associated with higher live birth and lower pregnancy loss rates (Brady et al., 2014). On the contrary, other authors have concluded that serum progesterone values greater than 20 ng/ml on the day of embryo transfer have a negative impact and were connected with lower rates of live birth and clinical pregnancy rates in donor recipient cycles (Kofinas et al., 2015). This controversy regarding optimal P4 values is likely due to variation in patient basal characteristics, including uterine dynamics and the quality of transferred embryos.

In summary, a measurement of serum progesterone has an important role in the prediction of the success of human embryo implantation. We propose that levels above 14.4 ng/ml during the embryo transfer are favorable for successful implantation after SFET. It was already shown that timing the transfer of a frozen embryo based on serial progesterone measurements is an effective strategy that results in higher pregnancy rates (Zhe et al., 2014). Similar approach could be applied in order to perform ET only when the P4 levels exceed the threshold value. However, further study is necessary to confirm the efficacy of the proposed cut-off for improvement of IVF procedures in natural cycles.

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