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Relationship between serum estradiol level, ultrasound follicle count, number of oocytes retrieved and their influence on IVF/ICSI treatment outcomes: a retrospective cross-sectional study

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ABSTRACT

This retrospective cross-sectional study investigates determinants on follicular development, oocyte retrieval and pregnancy outcome. It assessed the clinical practicability of monitoring parameters in relation to predict a successful treatment. Analysis of serum-estradiol (E2), sonographic follicle count, number of oocytes and optimizable parameters have therefore been carried out based on patient files from the IVF outpatient clinic at the Department of Gynecology, Obstetrics and Reproductive Medicine, Homburg/Saar, Germany. Equidirectional connection occurred between serum-E2, sonographic follicle count and number of oocytes ($p < 0.001$). There was no significant difference between sonographic and punctured follicle count ($p = 0.428$), but between sonographic/punctured follicle count and number of oocytes obtained ($p < 0.01$). Increasing endometrial thickness was associated with increasing serum-E2 ($p = 0.003$) and number of oocytes ($p < 0.001$), but not with the follicle count ($p > 0.05$). Additionally, age was inversely associated with sonographic follicle count and number of oocytes ($p < 0.001$) but not with E2 ($p > 0.05$). BMI, nicotine and stimulation protocol had no association with the observed parameters ($p > 0.05$). Mean differences in follicle numbers can be used for predicting expectable numbers of oocytes. Due to comparable numbers of follicles visualized on the day of ovulation induction and the number of follicles punctured, more emphasis should be placed in optimizing oocyte retrieval procedures.

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Introduction

According to the most recent committee opinion of the American Society of Reproductive Medicine (ASRM), infertility is defined as a 'disease, condition, or status' pertaining to the incapacity to attain a successful pregnancy due to factors related to patient history along with physical and laboratory findings, requiring some sort of medical intervention, for example in vitro fertilization (IVF)/ intracytoplasmatic sperm injection (ICSI). ASRM suggests, that the evaluation of infertility should be age-dependent. In cases where individuals engage in consistent, unprotected intercourse and there are no identifiable causes indicating impaired reproductive function in either partner, diagnostic investigation should commence after 12 months if the female partner is younger than 35 years old. Alternatively, if the female partner is 35 years of age or older, evaluation should begin after 6 months under similar circumstances [1]. Infertility impacts 10% of women aged 20-50 years and is frequently accompanied by notable physical and emotional burden [1]. Of note, the percentage of involuntarily childless couples has risen from 25 % to 32 % in recent years reflected by an increasing interest and use of assisted reproductive technologies (ART) [2,3].

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Estimating the success of ART/IVF treatment is a widely discussed field in the literature [2]. The most commonly used outcome for determination of success is life birth rate (LBR) [2,3]. A plethora of variables have been tested, including clinical factors (e.g., female age, weight, along with other measures of adiposity, cause of infertility, stimulation protocols) along with laboratory methods (e.g., vitrification of embryos) [2-4]. This has given rise to various validated and unvalidated tools, which may be used in clinical practice [4].

Ovarian stimulation is an important part of ART, leading to multifollicular development during one cycle [4]. The aim of multifollicular stimulation is to improve the chance of achieving good quality embryos and a successful treatment outcome by generating a good number of oocytes out of a wide range of follicles [5,6]. From a clinical perspective, great effort has been made in standardizing and improving ovarian stimulation and the timing of ovulation induction for more optimal reproductive outcomes to be achieved [5]. According to Steward et al., the retrieval of 6 to 15 oocytes after ovulation inductions leads to optimal live birth rates without the cost of ovarian hyperstimulation syndrome (OHSS), caused in cases more oocytes are retrieved [6].

In order to find balance between a good number of follicles developed and patient safety monitoring of ovarian stimulation plays a key role in ART [7]. Nowadays ultrasound is used routinely for the determination of the correct time of ovulation induction [7]. Measurement of the number and size of the leading follicles by ultrasound is important to evaluate optimal timing for ovulation induction with human chorionic gonadotrophin and to assess the risk of OHSS [8]. To bolster the outcomes of oocyte retrieval, some clinical and biochemical markers have been studied [9]. Oestradiol, an inherent sex steroid, has been proposed by some researchers, even though the exact role is yet not clear [9,10]. During ovarian stimulation multiple follicles develop. Most of oestradiol is produced by follicular granulosa cells [11]. Under ovarian stimulation multiple follicles develop with a higher number of granulosa cells differing from the normal menstrual cycle reaching a supraphysiological oestradiol production. Because of this link oestradiol is assessed to be a marker of follicular development as well as of quality of the follicle phase of the stimulated cycle [9]. Strawn et al. showed that the determination of oestradiol concentrations at the day of ovulation induction provided no additional benefit regarding clinical pregnancy rates and the number of oocytes retrieved [10]. On the contrary, Li et al. underlined the importance of preovulatory serum oestradiol levels as valuable marker for predicting premature ovulation in unstimulated cycles in IVF [11]. In essence, a clear consensus on the use of oestradiol for stimulation monitoring has not been achieved, especially given the fact that some authors consider it expensive and inconvenient for the patients [12,13].

The present study evaluated factors influencing follicular development, oocyte retrieval and ART outcome. Our objectives were to: (1) analyze the relationship between serum-E2 level, sonographic follicle count and number of oocytes received; (2) assess the monitoring system of stimulated cycles; (3) identify optimizable predictive parameters for achieving pregnancy.

Materials and Methods

Inclusion and exclusion criteria

The inclusion criteria of this retrospective cross-sectional study were:

- Patients who underwent IVF/ICSI at the Department of Gynecology, Obstetrics and Reproductive Medicine in Homburg/Saar, Germany between March 2012 and April 2022
- Both single and multiple cycle attempts were included.
- Availability of patient records in the IVF clinic at the time of data collection between March and May 2022

Exclusion criteria constituted:

- Procedures other than IVF/ICSI (e.g. intrauterine insemination)
- Incomplete patient records (for the data collection) or no available records during the time of data collection.

Participants, treatment

We included 235 patients and 411 ART cycles. Standard IVF/ICSI treatment procedures were followed [13]. Patients underwent individually adapted stimulation protocols. The GnRH antagonist protocol was used as standard protocol while the GnRH agonist protocol remained for patients with endometriosis or stimulation-failure (more than 3 stimulation cycles without fulfilling the criteria for ovulation induction). Patients within the antagonist protocol received 150 IU follitropin alfa from day three/four up to day 13/14 of the menstrual cycle as well as 0.25 mg of cetrorelix acetate from day seven up to day 13/14. Follicular development was evaluated three times within a stimulation cycle: At the beginning of stimulation, on day five/six and close to the day of ovulation induction. For examination patients were in lithotomy position. Examinations were performed by different investigators. The ovaries were scanned in longitudinal and frontal planes. All follicles were counted (regardless of their size) and endometrial thickness was recorded at the level of greatest expansion. If three or more follicles larger than 17 mm occurred, ovulation was induced with choriogonadotropin alfa followed by transvaginal ultrasound-guided follicular puncture 36 hours afterwards.

Regarding baseline estradiol measurement, this was conducted as part of routine care around the day of ovulation of induction. Serum monovette (S-Monovette Serum-Gel®, Sarstedt Nümbrecht, Germany) were used for venipuncture. Electrochemiluminescence-immunoassay-kits (COBAS®e801, Roche-Diagnostics GmbH, Mannheim, Germany) were used for measuring the serum-E2 at the central laboratory of the Department of Clinical Chemistry and Laboratory Medicine of the Faculty of Medicine Homburg.

Variables

We extracted the following data from available patient files in a predefined Microsoft Excel file: 1) baseline and anthropometric data (age at follicle puncture, height (in centimeters), weight (in kilograms), body mass index (BMI), previous pregnancies, smoking status), 2) parameters of cycle monitoring (number of follicles, E2 and endometrial thickness around the day of ovulation induction), 3) parameters of the follicle puncture (number of punctured follicles, oocytes received) and the outcome. Focus was placed on the number of follicles recorded in 2D-US and E2-levels collected by venous-blood-serum around the day of ovulation induction.

Statistical analysis

Normality of distribution was evaluated using the Kolmogorov-Smirnov test along with visual examination of the data. Given that all data were not normally distributed, we used median (range) for data presentation, along with non-parametric statistics for group comparisons (Mann-Whitney test for 2 groups, Kruskal-Wallis for more than 2 groups). For qualitative variables, we used absolute number (percentages) as summary statistics.

To examine the association of studied parameters (monitoring parameters, clinical parameters – confounders, measuring days) we fitted univariable linear regression models (dependent variables were all continuous) and non-parametric tests were performed. Microsoft Excel and IBM-SPSS 29 were used for data analysis. Statistical significance was defined as $p < 0.05$.

Results

Patient characteristics

Patient characteristics are shown in Table 1. As far as pregnancy outcomes are concerned, seven times (1.7%) there was a documented abortion or extrauterine pregnancy (EUG). Biochemical pregnancy occurred in 5.4% and was defined as detectable beta-hCG increase above 10 IU/l. An intact clinical pregnancy without knowledge of the further course, was achieved in 10.2% and was defined as sonographically or histological detectable gestational sac. Deducting miscarriages and EUGs, total pregnancy rate (biochemical and clinical pregnancies) was 15.6%.

Table 1. Baseline characteristics of our sample (n = 235 patients / 411 cycles)

Parameter	Median (Range)/ Number (%)	Missing values (%)
Age (years)	34 (19-44)	4 (1%)
Height (cm)	166 (150-184)	9 (2%)
Weight (kg)	75 (47-178)	12 (3%)
BMI (kg/m^2)	27 (18-56)	13 (3%)
Nicotine consumption	59 (14%)	7 (2%)
Infertility type		0 (0%)
Primary	246 (60%)	
Secondary	165 (40%)	
ART treatment		4 (1%)
IVF	92 (22%)	
ICSI	315 (77%)	
Stimulation protocol		26 (6%)
Agonist	80 (20%)	
Antagonist	305 (74%)	

ICSI: intracytoplasmic sperm insemination, IVF: in vitro fertilization

Monitoring parameters

Linear regressions of clinical and laboratory parameters with outcome measures are shown in Table 2. Increasing endometrial thickness was associated with a statistically significant linear increase in serum-E2 as well as oocytes retrieved ($R^2 = 0.025$, $p = 0.003$; $R^2 = 0.03$, $p < 0.001$, respectively). The number of follicles sonographically detected demonstrated no association with endometrial thickness ($R^2 = 0.009$, $p = 0.067$). Serum-E2 increased with increasing number of follicles monitored ($R^2 = 0.31$, $p < 0.001$) and also with increasing number of oocytes retrieved ($R^2 = 0.224$, $p < 0.001$). The number of oocytes demonstrated a positive association with increasing sonographic follicle count ($R^2 = 0.283$, $p < 0.001$). Linear regression also showed a significant association in punctured follicle count with increasing numbers of sonographic follicles at monitoring as shown in Table 2 ($R^2 = 0.396$, $p < 0.001$).

Confounding variables

Regression analysis of E2 according to age at follicle puncture indicated no significant relationship ($R2 = 0$, $p > 0.05$) (Table 2). Increasing age was associated with a statistically significant linear decrease in sonographic follicle count ($R2 = 0.053$, $p < 0.001$) as well as oocytes received ($R2 = 0.048$, $p < 0.001$). There was no significant association between levels of E2 and age ($R2 = 0$, $p > 0.05$). No significant relationship could be found between E2, sonographic follicle count and oocytes retrieved according to the BMI ($R2 = 0.004$, $p > 0.05$; $R2 = 0.005$, $p > 0.005$; $R2 = 0.001$, $p > 0.05$) and smoking status ($R2 = 0.009$, $p > 0.05$; $R2 = 0.001$, $p > 0.05$; $R2 = 0.003$, $p > 0.05$). There was no significant association between the outcome and E2

(R2 = 0, p > 0.05), sonographic follicle count (R2 = 0.004, p > 0.05) and oocytes retrieved (R2 = 0, p > 0.05).

Measuring days

A later measuring day was associated with a statistically significant linear increase in serum-E2 (R2 = 0.028, p < 0.001) and endometrial thickness (R2 = 0.036, p < 0.001) (Table 2). Sonographic follicle counts according to a later measurement day indicated no relationship (R2 = 0, p > 0.05).

A later measuring day was associated with a statistically significant linear increase in serum-E2 (R2 = 0.028, p < 0.001) and endometrial thickness (R2 = 0.036, p < 0.001). The alternating follicle measurement day was negligible as a potential influencing factor.

Table 2. Linear regression analysis between serum-E2, sonographic follicle count, punctured follicle count, number of oocytes received and endometrial thickness and clinical parameters. R^2 corresponds to the coefficient of determination.

	Serum E2 level	Sonographic follicle count	Number of oocytes retrieved	Endometrial thickness
Serum E2 level	-	$R^2 = 0.31$ $p < 0.001$	$R^2 = 0.224$ $p < 0.001$	$R^2 = 0.025$ $p = 0.003$
Sonographic follicle count	$R^2 = 0.31$ $p < 0.001$	-	$R^2 = 0.283$ $p < 0.001$	$R^2 = 0.009$ $p = 0.067$
Number of oocytes retrieved	$R^2 = 0.224$ $p < 0.001$	$R^2 = 0.31$ $p < 0.001$	-	$R^2 = 0.030$ $p < 0.001$
Endometrial thickness	$R^2 = 0.025$ $p = 0.003$	$R^2 = 0.009$ $p = 0.067$	$R^2 = 0.030$ $p < 0.001$	-
Measuring day	$R^2 = 0.028$ $p < 0.001$	$R^2 = 0$ $p = 0.82$	-	$R^2 = 0.036$ $p < 0.001$
BMI	$R^2 = 0.004$ $p = 0.25$	$R^2 = 0.005$ $p = 0.162$	$R^2=0.001$ $p = 0,646$	-
Nicotine consumption	$R^2 = 0.009$ $p = 0.079$	$R^2 = 0.001$ $p = 0.526$	$R^2 = 0.003$ $p = 0.317$	-
Stimulation protocol	$R^2 = 0$ $p = 0.96$	$R^2 = 0.003$ $p = 0.295$	$R^2 = 0$ $p = 0.925$	-
Age	$R^2 = 0$ $p = 0.76$	$R^2 = 0.053$ $p < 0.001$	$R^2 = 0.048$ $p < 0.001$	-

Discussions

Based on a linear regression model increasing endometrial thickness was found to be associated with increasing serum-E2 levels and oocytes received. Mahutte et al. obtained similar results [14]. Rehman et al. showed that increasing serum-E2 conditioned optimal endometrial thickness, associated with the highest fertilisation and clinical pregnancy rates in their cohort [15]. Dietterich et al. expounded contrary results. Their analysis presented no differences regarding peak E2 levels during ovulation induction and number of oocytes obtained in groups with differing endometrial thickness [16]. Based on the evaluated data in our analysis, no significant association was observed between the sonographic follicle count and endometrial thickness.

Contrary, Mahutte et al. showed higher pregnancy rates, serum-E2 levels, number of preserved oocytes and number of mature oocytes in patients with higher endometrial thickness (> 9 mm) [14]. Higher follicle counts associated with higher endometrial thickness can be explained by more successful follicle stimulation with correspondingly higher endometrial thickness [17]. Limiting the comparability of our results, as well as the literature references [16-19], was the variability of the sonographic thickness measurements carried out by different examiners with different experiences and techniques. It would therefore be useful to conduct another more objective study with one designated examiner using standardized measurement protocols.

Patients with higher serum-E2 levels showed increasing numbers of follicles by ultrasound and oocytes retrieved. Several independent studies draw concordantly the same conclusion [18]. This can be explained by the fact that E2 was primarily produced by the granulosa cells of the ovarian follicles [11]. Consequently, in multifollicular maturation under hormonal stimulation treatment a greater number of granulosa cells could be found, which led to supraphysiological serum-E2 levels via increased E2 production. The possibility of monitoring serum-E2 as a predictive factor regarding the expected number of follicles

for follicle puncture was confirmed and for clinical practice the option of using E2 to make predictive statements about an expectable number of follicles and the optimal time of ovulation induction can be postulated. In future research it is important to analyze influences on the basal serum-E2 level of the patients as well as the general individual hormonal constitution due to concomitant diseases or pathological changes in hormonal balance and how that can be included to make more exact predictions regarding the number of follicles. Kapoor et al concluded an increasing meiotic capacity of the oocytes with increasing peak E2 and thus decisively improved oocyte maturation [20].

In their retrospective study, Morales et al. succeeded in giving a value of 300 pg/ml per expected oocyte retrieved, which is the most exact value currently found in literature with regard to the question investigated [19]. In summary of our results, it can be assumed that expected numbers of oocytes can be narrowed down using serum-E2, but only with limited significance and accuracy. Against this background, the question of clinical relevance of serum-E2 for determining expected numbers of oocytes arises. The psychological consequences of raising hopes for high numbers of expectable oocytes based on hormone levels, that may turn out to be significantly lower, should be considered. The aim of our study was to generate mean differences between the sonographic follicle count, the number of punctured follicles and the oocytes received in order to derive predictive statements for clinical practice.

There was no significant difference between sonographic follicle count and the number of punctured follicles, whereas there were significant differences between the number of punctured follicles and the number of retrieved oocytes with a mean difference of 3.28 pieces and even 3.63 pieces between the sonographic follicle count and the number of oocytes. Wang et al. showed that patients in whom the number of retrieved oocytes was significantly lower than the number of visible follicles, had an existing infertility over a longer period of time and higher levels of follicle stimulation hormone, indicating poorer ovarian reserve with an influence on the success of oocyte retrieval [20]. Furthermore, they registered higher levels of luteinizing hormone and lower E2 and progesterone levels on the day of ovulation induction, indicating inadequate follicular development with insufficient hormone production by granulosa cells. Wang et al identified inadequate oocyte production more often in patients with ovarian dysfunction [20].

Our work showed no significant association between the outcome and serum-E2, sonographic follicle count and oocytes retrieved. Chotboon et al. draw the conclusion that although serum-E2 levels in combination with the number of mature metaphase ll-oocytes was a predictor of oocyte maturation and fertilization probability, as an isolated factor no conclusions regarding the probability of pregnancy occurrence can be derived from it [21]. Similarly, Kyrou et al. found no significant differences in continued pregnancies in groups with different levels of serum-E2 [22]. Some observers explained this by detrimental effects of elevated E2 levels on endometrial receptivity, caused by reduced endometrial blood flow and increased uterine contractility but the exact mechanism remains unknown [23]. Li et al. drew a different conclusion [24]. The oocytes retrieved, implantation rates as well as the clinical pregnancy rate, increased with increasing concentrations of E2 up to 5,000 pg/ml. The parameters worsened when E2 levels exceeded 5,000pg/mL. According to the study, the optimal serum-E2 level was estimated between 1,000 to 3,148 pg/ml [24].

There was no significant association between sonographic follicle and the outcome in our analysis. In contrast, Tan et al. found the number of follicles before the day of hCG injection considered as an independent predictor of clinical pregnancy rate [25]. The follicular diameter therefore appears to be an indicator of oocyte maturity and an assessment parameter for oocyte quality as proposed by Hunter et al. [26].

In our work, the distribution of oocytes did not differ significantly between the groups with different outcomes. Cai et al. confirmed our conclusion, even though they failed to find significant differences regarding pregnancy rates and birth rates in groups with different numbers of oocytes received [27]. The graphical relationship between oocyte retrieval and pregnancy rate was almost identical to the relationship between oocyte retrieval and the likelihood of obtaining two embryos with good quality. They concluded the number of good quality embryos as main factor influencing pregnancy rates [27]. Thus, patients with more than eight oocytes had the highest probability of receiving two good quality embryos and the highest probability of achieving a pregnancy [27].

Thaker et al. postulated an optimal range of oocyte numbers and defined the goal of IVF treatment as generating an optimal number of oocytes rather than the maximum achievable [28]. In contrast to our results, they identified a significant positive correlation between number of oocytes retrieved and the IVF outcome. Positive IVF outcomes were significantly higher in groups of intermediate number of oocytes (6-10 oocytes) than in groups with a lower number of oocytes (< 6 oocytes) [29,30]. However, no additional benefit was found between medium and high egg counts (> 10 eggs). A further increase in oocyte numbers beyond medium ranges, given the risk of overstimulation [29]. Contrasting results to our findings could be explained by the fact, that in our study only the correlation between number of oocytes and the overall pregnancy rate was observed but not the clinical pregnancy rate.

Our results showed a counteracting relationship between age and E2 levels and sonographic follicle count, coincided with the results of the research group around

[11]. Our hypothesis was supported by them showing decreasing serum-E2 and follicle numbers with increasing age, which can be attributed to a decreasing ovarian reserve resulting in a reduced response to hormonal stimulation and an equally reduced multifollicular maturation [30]. Aizer et al. demonstrated a negative correlation between age and oocyte numbers beyond the age of 35 years [31]. In our analysis, there was no association between BMI and the parameters studied. In agreement with a retrospective paper by Friedler et al., patients' BMI at the time of ART treatment did not influence serum-E2 levels and the number of eggs retrieved [32]. Contrary, Zhou et al. revealed a negative correlation between increasing BMI and number of oocytes retrieved [33].

Our study failed to identify differences between non-smokers and smokers in terms of serum-E2 level, sonographic follicle count and number of oocytes retrieved. De Angelis et al. found no adverse effect of nicotine use on ovarian follicle development (no significant differences in growing follicles) [34] similar to Ozbakir et al., who also showed no negative effects on the follicle count and the number of oocytes retrieved [35]. In contrast to our results, Galanti et al. found a reduced ovarian function and a negative impact on IVF results through an increase in ROS production [36]. Based on the previous notion, the general recommendation is to discontinue tobacco consumption as a risk factor for several secondary health diseases, and especially with regard to teratogenic effects through transplacental damaging effects on the fetus [37].

There were no significant differences in serum-E2 level, sonographic follicle count and number of oocytes retrieved between the stimulation protocols studied. Lai et al. confirmed our findings [38]. Another article reported significantly higher pregnancy rates, a lower E2/oocyte ratio for the agonist protocol but showed no differences between the different protocol groups for number of oocytes [39].

As far as strengths of our study are concerned, we included a large number of cases including both IVF and ICSI, representing a broad aspect of the infertile population. A limitation of the study was due to its retrospective character that missing data could not be completed. Additionally, the inconsistent measurement times of E2, follicle count and endometrial thickness adds bias to the analysis. Monitoring and follicle puncture were carried out by different doctors with different experience levels and individual procedures, which meant that it was not possible to objectify the procedure of the measurement as well as the results.

Conclusions

We revealed a correlation between E2, sonographic follicle count and number of oocytes received. This underlines the possibility to predict an expectable number of oocytes due to mean differences in follicle counts. Due to comparable numbers of follicles visualized on the day of ovulation induction those punctured, the future goal does not seem to be to optimize follicle visualization and puncture but optimize the oocyte retrieval procedure. The study revealed age as the only modifiable patient characteristic with a significant impact on the studied cycle parameters.

Contributions

Conceptualization, S.F. and C.H.; methodology, T.S. and C.H.; software, S.-L.B.; validation, S.F. and E-F.S.; formal analysis, G.W. and C.H.; investigation, S.F.; resources, E-F.S., S.F.; data curation, S.F. and C.H.; writing—original draft preparation, C.H.; writing—review and editing, S.F., E-F.S., B.H.H., G-P. B, M. N and R.M.S.; visualization, S.F. and C.H.; supervision, S.F. and E-F.S.; project administration, S.F. and E-F.S.

Compliance with ethical standards

Any aspect of the work covered in this manuscript has been conducted with the ethical approval of all relevant bodies and that such approvals are acknowledged within the manuscript. Informed consent was obtained from all subjects involved in the study.

Conflict of interest disclosure

There are no known conflicts of interest in the publication of this article. The manuscript was read and approved by all authors.

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