Prognostic factors for clinical pregnancy using *in vitro* fertilization / intracytoplasmic sperm injection and embryo transfer procedures

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Objective. To evaluate the prognostic factors for clinical pregnancy using *in vitro* fertilization (IVF) / intracytoplasmic sperm injection (ICSI) and embryo transfer (ET) procedures for the period 2000–2003.

Study design. A retrospective study of 311 *in vitro* fertilization cycles, performed on 252 women in the "Vaisingumo klinika" fertility clinic in Lithuania during 2000–2003.

Results. After 311 cycles the clinical pregnancy rate was 29.6%. Pregnancy rate was significantly higher for women with type I endometrial pattern (38.7%) *versus* 7.8% for those with type II endometrial pattern ($p < 0.0001$). The rate for women younger than 35 years was higher than for older ones (36.1 and 19.5 percent, respectively, $p = 0.002$). The long ovary stimulation protocol had a significant influence on clinical pregnancy rate in comparison with the short protocol or the protocol using antagonists ($p = 0.028$). 220 cycles with perfect embryo transfer resulted in 75 clinical pregnancy cases (pregnancy rate 34.1%). Perfect quality of embryo transfer, compared with the poor quality transfer, had the largest impact on the probability of clinical pregnancy (OR 5.34, 95% CI 1.45–19.7). The long stimulation protocol, if compared with the short one, was also a significant predictor (OR–2.31, 95 percent CI 1.19–4.47). Every embryo transferred additionally increased the probability of clinical pregnancy 2.14-fold (95 percent CI 1.49–3.07). The age of 35 years and over decreased the probability of clinical pregnancy.

Conclusions. Type I endometrium, endometrial thickness over 11 mm, age below 35 years, perfect quality of embryo transfer and long stimulation protocol were significant prognostic factors for a higher clinical pregnancy rate using ART.

Key words: IVF, ICSI, ET, prognostic factors, Lithuania

INTRODUCTION

Assisted reproductive techniques (ART) such as *in vitro* fertilization, intracytoplasmic sperm injection and embryo transfer are the most effective methods of infertility treatment for some indications (1).

This kind of treatment is rather new in Lithuania (started in 1998) (2), therefore it is important to evaluate the prognostic factors influencing successful treatment outcomes.

Many studies have identified several factors that predict higher probability of pregnancy after ART. These are female age (3), indications for a procedure (4), the quality of embryo transfer (5, 6), endometrial pattern and its thickness on the day of hCG administration (7–14), the type of superovulation induction and body mass index (BMI) (15–17). The relation between endometrial pattern, its thickness and implantation possibility is discussed by many authors, but still there is no final conclusion about the influence of these factors on pregnancy rate (7).

Numerous studies have reported that a thicker endometrium with type I pattern ('triple line') has a positive influence on pregnancy rate (8, 9, 18). Thin endometrium has been shown to be a prognostic indicator of pregnancy only in a case of female indications for infertility (10), while some studies have indicated that endometrial thickness and pattern are not predictive in ART (11–13, 19). Some authors (14, 20, 21) have suggested that type II pattern of endometrium (hyperechogenic, homogenic) is associated with a poor IVF-ET outcome.

Female age, without any doubt, is one of the most important factors in ART prediction. The more advanced age, the higher doses of FSH are needed to induce superovulation and less oocytes are usually obtained (3). Endometrium receptivity also decreases with age. Moon (3) has reported a significant negative linear

correlation between female age and the numbers of oocytes retrieved and embryos transferred, and cumulative embryo score. Clinical pregnancy rates were significantly decreased with advancing age.

The French researcher Wittemer (15) investigated female BMI influence on different parameters of IVF procedure and outcome. When BMI was equal or higher that 25 kg/m², more gonadotropin ampoules were consumed and less oocytes were collected. No significant difference has been found in the clinical pregnancy rate between different BMI groups. Similar findings were described by Lashen (16), whereas Loveland (17) indicated that when BMI was higher than 25 kg/m² the pregnancy rate was significantly lower.

Tomas (5) has analyzed the influence of embryo transfer quality in pregnancy prediction. According to this study, easy or intermediate transfers resulted in a 1.7-fold higher pregnancy rate than difficult transfers (P < 0.0001; 95% confidence interval: 1.3–2.2).

The purpose of the present study was to evaluate the prognostic factors for clinical pregnancy using *in vitro* fertilization/intracytoplasmic sperm injection and embryo transfer procedures carried out during 2000–2003 in "Vaisingumo klinika" ("Fertility clinic").

MATERIALS AND METHODS

In total, 252 couples treated by IVF or ICSI and ET between July 2000 and December 2003 in the fertility clinic "Vaisingumo klinika" in Lithuania were selected for this retrospective analysis. Cycles without fresh embryo transfer and epidydimal or testicular sperm were excluded. Altogether, 311 cycles were included for analysis.

The women's age was estimated at the time of ART. BMI was calculated by dividing the weight in kilograms by the height in meters squared (kg/m²).

An ultrasound scan was performed by using SONOLINE Adara ultrasound with 7.5 MHz vaginal probe; endometrium thickness and type were evaluated on the day of hCG injection.

Endometrium was estimated as type I if three lines were defined and as type II if it was homogenic hyperechogenic (21).

Endometrial thickness was measured by longitudinal scanning of the uterus on the frozen image using electronic callipers placed at the endometrium–myometrium interface at the level of the fundus (10).

Ovary stimulation protocol was chosen according to female age and indication for ART. To achieve the controlled ovary hyperstimulation, we used gonadotropin hormone agonists (GnRH a): Goserelinum (Zoladex 3.6 mg, Astra Zeneca), Triptorelinum (Diphereline 3.75 mg, Beaufour Ipsen), Buserelinum (Suprecur®, Aventis Pharma), Triptorelinum (Decapeptil 0.1 mg, Beaufour Ipsen), antagonists (GnRH an) Cetrorelixum (Cetrotide, Serono), and gonadotropins Follitropinum alfa (Gonal F, Serono), Follitropinum beta (Puregon®, Organon), rLH (Luveris, Serono).

The following ovary stimulation protocols were applied:
• "long" – starting GnRH agonist in the middle of the luteal phase of the previous menstrual cycle and from either the 3rd, 4th or 5th day of the following menstrual cycle daily rFSH was added (the so-called "golden standard") (22);
• "short" – starting GnRh agonist in the beginning of the follicular phase, and from the 2nd or 3rd day of the same menstrual cycle daily rFSH was added (22);
• "with antagonists" – in the beginning of the follicular phase rFSH was started, and when the dominating follicle reached 14 mm in diameter GnRh was added (23).

The decision to prescribe one of themy was made taking into account the age of patients, endometriosis status, male factor infertility, poor ovarian response during previous ART cycles. The protocol "with antagonists" was used more often in cases with the diagnosed polycystic ovary or chronic anovulation syndrome to avoid the ovarian hyperstimulation syndrome (OHSS).

The quality of embryo transfer was evaluated according to the methodology accepted in "Vaisingumo klinika". The methodology was designed according to results of the study published by Alvero et al. about the negative influence of blood in the transfer catheter (6):
• perfect quality – when a soft, flexible Dolphin catheter was used and it went through the cervical canal easily, without any resistance. After the procedure no blood or mucus were found on the catheter;
• medium quality – when a soft, flexible Dolphin catheter was used, and it went through the cervical canal with difficulty, several attempts were needed. After the procedure no blood or mucus were found on the catheter;
• poor quality – when it was necessary to change the flexible Dolphin catheter with a stiffer Dolphin catheter. After the procedure blood or mucus could be found on the catheter.

Only clinical pregnancies were evaluated. The pregnancy was determined by serum hCG test and was confirmed by finding a gestational sack in the uterus by ultrasound examination.

There are some differences in the total numbers of ART cycles in tables because of the lack of data in some cases.

The analysis of results was made using the Statistics Package for Social Sciences SPSS 8.0 and MedCalc 7.1.0.1. The differences between the groups described by categorical variables were analyzed using the c^2 test, while for continous variables analysis of variance (ANOVA) was used. The chosen level of statistical significance was 5% (the differences were considered statistically significant when the p value was less than 0.05). A stepwise multiple logistic regression analysis with backward elimination was performed to evaluate the significance of factors important for ART outcome: endometrial pattern, endometrial thickness, number of transferred embryos, female age, quality of embryo transfer,

Table 1. **Pregnancy rate according to endometrial pattern**

Pregnancy	Endometrial pattern		
	Type I n (%)	Type II n (%)	Total n (%)
Yes	84 (38.7)	7 (7.8)	91 (29.6)
No	133 (61.3)	83 (92.2)	216 (70.4)
Total	217	90	307

Dependence between pregnancy and endometrial pattern was analyzed using Chi² test. The differences were statistically significant (p < 0.05).

Table 2. **Pregnancy rates according to endometrial thickness**

Pregnancy	Endometrial thickness, mm			
	< 9 n (%)	9–11 n (%)	> 11 n (%)	Total n
Yes	15 (22.1)	43 (27.4)	34 (40.5)	91
No	53 (77.9)	114 (72.6)	50 (59.5)	217
Total	68	157	84	309

Dependence between pregnancy and endometrial thickness was statistically significant (p < 0.05).

Table 3. **Pregnancy rate according to endometrial thickness and pattern type**

Endometrial pattern	Endometrial thickness, mm				
	< 9 n (%)	9–11 n (%)	> 11 n (%)	Total n (%)	P
Type I	11 (27.5)	39 (35.8)	34 (50)	84 (38.7)	0.046
Type II	4 (14.3)	3 (6.5)	0 (0)	7 (7.8)	0.212
Total	15 (22.1)	42 (27.1)	34 (41.0)	91	

Table 4. **Pregnancy rates according to female age groups**

Pregnancy	Age groups, years		
	< 35 n (%)	≥35 n (%)	Total n
Yes	69 (36.3)	23 (19.7)	92
No	121 (63.7)	94 (80.3)	215
Total	190	117	307

P < 0.05.

ovary stimulation protocols, BMI. Due to missing variables in some cases the total number of cycles presented in univariate and multivariate analysis differed.

RESULTS

After 311 IVF and ICSI and ET cycles clinical pregnancy was diagnosed in 92 cases and the pregnancy rate was 29.6%.

Type I endometrial pattern had a significant influence on the pregnancy rate: 38.7% of all cycles resul-

Table 5. **Pregnancy rates according to female body mass index (BMI)**

Pregnancy	BMI, kg/m²			
	< 20 n (%)	20–25 n (%)	> 25 n (%)	Total n
Yes	12 (33.3)	70 (30.2)	10 (24.4)	92
No	24 (66.7)	162 (69.8)	31 (75.6)	217
Total	36	230	41	307

Dependence between pregnancy and BMI was not statistically significant (p = 0.669).

Table 6. **Pregnancy rates according to ovary stimulation protocol**

Pregnancy	Stimulation protocol			
	Short n (%)	Long n (%)	With antagonists n (%)	Total n
Yes	22 (21.6)	54 (37.0)	16 (26.7)	92
No	80 (78.4)	92 (63.0)	44 (73.3)	216
Total	102	146	60	308

Dependence between pregnancy and stimulation protocol was statistically significant (p < 0.05).

ted in clinical pregnancy, *versus* only 7.8% when type II endometrial pattern was assessed (p < 0.0001) (Table 1). The mean endometrial thickness on the day of hCG administration in the clinical pregnancy group was 10.6 mm (95% CI 10.2–10.9), and for those who did not conceive it was 10.1 mm (95% CI 9.9–10.4, p = 0.044). In the majority of all cases treated with ART (50.8%), endometrial thickness was between 9 and 11 mm (Table 2). However, when pregnancy rate dependen-

Table 7. **Pregnancy rates according to the quality of embryo transfer**

Pregnancy	Quality of embryo transfer			
	Poor n (%)	Medium n (%)	Perfect n (%)	Total n
Yes	3 (9.7)	14 (25.0)	75 (34.1)	92
No	26 (89.7)	42 (75.0)	145 (65.9)	213
Total	29	56	220	305

Dependence between pregnancy and quality of embryo transfer was statistically significant (p < 0.05).

ce only on endometrial thickness group was analysed, the largest proportion of clinical pregnancies (40.5%) was diagnosed when endometrial thickness was more than 11 mm. When the endometrium pattern and endometrial thickness were analysed together, results were not statistically significant, but the combined effect of type I pattern and thickness over 11 mm resulted in a 50% pregnancy rate (p < 0.05) (Table 3).

Table 8. **Pregnancy rate dependence on various prognostic factors using logistic regression analysis**

Prognostic factors	OR	95% CI	
Type II endometrial pattern	0.17	0.07	0.40
Number of embryos transeferred	2.14	1.49	3.07
Female age, 35 years or more	0.54	0.29	1.00
Perfect quality of embryo transfer	5.34	1.45	19.70
Long stimulation protocol	2.31	1.19	4.47

Clinical pregnancy rates by age groups are presented in Table 4. The rate for women younger than 35 years was nearly twice higher than that for those aged 35 years or over (36.1% and 19.5%, respectively, p = 0.002).

The majority of women (74.9%) were within the normal range of BMI, *i.e.* it was between 20 and 25 kg/m². According to our data, pregnancy rates did not differ among underweight (BMI below 20 kg/m²), normal and overweight or obese (above 25 kg/m²) women (Table 5).

The long ovary stimulation protocol had a statistically significant influence on ART outcome in comparison with the short protocol or the protocol using antagonists (p = 0.028, Table 6).

Measuring according to the methodology accepted in "Vaisingumo klinika", most often embryo transfer quality was estimated as perfect. Overall, 220 cycles with a perfect embryo transfer resulted in 75 clinical pregnancy cases, the pregnancy rate being 34.1%. The corresponding rates in groups with a poor and medium quality embryo transfer were significantly lower (p = 0.021, Table 7).

The importance of all factors was evaluated using logistic regression analysis with backward elimination (Table 8). The perfect quality of embryo transfer, compared with poor quality transfer, had the greatest statistically significant impact on the probability of clinical pregnancy, its OR being 5.34, with 95% confidence intervals (CI) 1.45–19.7. The long stimulation protocol, if compared with the short one, was also a significant positive independent factor for clinical pregnancy, with OR–2.31, 95 percent CI 1.19–4.47. Every additional transferred embryo increased the probability of clinical pregnancy 2.14-fold, 95 percent CI 1.49–3.07. Type II endometrial pattern had a significant negative influence on pregnancy, OR-0.17 (95% CI 0.07–0.40). The age of 35 years and over decreased the probability of clinical pregnancy.

DISCUSSION

Vera et al. (9) indicated that endometrial thickness and pattern, taken together, offer a high prognostic value for the outcome of an IVF-ET cycle. When both variables, thickness and pattern, were considered, patients with endometrial pattern type I and thickness >11 mm had the probability of clinical pregnancy and of delivery of 43.8% and 25% respectively, which was statistically significant (p < 0.03).

Findings from our study support these data and show that the combined effect of type I pattern and thickness over 11 mm resulted in a 50% pregnancy rate (p < 0.05).

Mean endometrial thickness for women who conceived and did not conceive in this study did not differ significantly (11.8 ± 1.8 mm and 10.6 ± 1.7 mm, respectively). No pregnancies occurred when endometrial thickness was less than 9 mm (9). The findings of our study proved that endometrial thickness and the type of endometrial pattern were independent significant predictors of clinical pregnancy. Although the pregnancy rate was highest among those with endometrial thickness over 11 mm, 15 pregnancies (22.1%) occurred in women with its thickness below 9 mm.

Check et al. (24) analyzed the influence of endometrial pattern type when the endometrial thickness was over 10 mm on the prognosis of the performed procedures. They found that only one of 22 patients with the hyperechogenic pattern achieved chemical pregnancy and none clinical pregnancy. In contrast, 67 from 251 (26.7%) patients conceived with other echo patterns (c² analysis 5.9, df = 1, P = 0.01). Our data are similar to those, the pregnancy rate in type I endometrial pattern being even higher. Therefore, we can state that type I endometrial pattern on the day of hCG administration was a statistically significant prognostic factor of IVF / ICSI outcome.

Although data from our and other studies showed a positive influence of thicker endometrium on pregnancy rate, Sundstrom (25) has presented an example when clinical pregnancy was achieved despite the endometrial thickness being just 4 mm on the follicle aspiration day. He suggested not to terminate the ART cycle because of the thin endometrium, particularly if other parameters such as follicle growth and serum estradiol concentration are favourable.

Rinaldi el al. (10) analyzed 109 patients for whom IVF was used due to female factor and 49 patients who had ICSI because of a male factor. In IVF cases the pregnancy rate was higher when endometrial thickness was over 10 mm (32 from 79; 41%) compared with endometrial thickness below 10 mm (5 from 30; 17%, p = 0.03). Whereas in ICSI the statistically significant pregnancy rate dependence on endometrial thickness was not detected: when endometrial thickness was over 10 mm, 13 out of 42 got pregnant (31%), and when it was below 10 mm, 3 out of 7 got pregnant (43%). Thin endometrium was a prognostically significant factor only when IVF was indicated due to a female factor. Unfortunately, we have to admit that when type II endometrial pattern on the day of hCG administration is detected we have no means to change this situation in the same superovulation induction cycle. Besides, we cannot predict the reaction of the endometrium to ovary stimulation. Administration of additional estrogens can be helpful in cases of a thin endometrium (26).

Wittemer et al. (15) explored the influence of BMI on pregnancy rate and established that for both over-

weight and very underweight women this factor had a negative impact on IVF parameters and result, although no statistically significant difference between BMI groups was found. Our data do not indicate that BMI would have a significant impact on pregnancy rate. In this study we did not evaluate the quantity of gonadotropins, the length of stimulation, difference between the quantities of oocytes received, miscarriage rates in different BMI groups. For a better evaluation of BMI influence on ART outcomes all these factors should be examined together.

Tomas et al. (5) analyzed the influence of embryo transfer quality on pregnancy rate. These researchers indicated that when the procedure was estimated as perfect or of medium quality, the pregnancy rate was 1.7-fold higher (p < 0.0001; 95% CI 1.3–2.2) if compared with a poor quality. We found that with the perfect quality embryo transfer clinical pregnancy was achieved five times more often than in cases of poor quality. The pregnancy rate for medium quality did not differ significantly from the poor quality pregnancy rate.

In our study, the pregnancy rate for women in the younger age group was higher. We linked the age with the thickness of endometrium. Still, the possibility for younger women to get pregnant was influenced not only by endometrium type, but also by the number of oocytes got during the procedure, which generally is bigger. In that case we get a bigger quantity of embryos and thus are able to make embryo selection and improve the results of the procedure. The most frequent indication for ART in young women is tubal occlusion, *versus* more complex indications for older women, and this most probably also contributes to the higher pregnancy rate in younger women (27).

CONCLUSIONS

Type I endometrium and endometrial thickness over 11 mm on the day of hCG administration, age below 35 years, perfect quality of embryo transfer and long stimulation protocol were significant prognostic factors for a higher clinical pregnancy rate using ART.

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KLINIKINIO NËÐTUMO PROGNOSTINIAI VEIKSNIAI TAIKANT APVAISINIMÀ *IN VITRO* (INTRACITOPLAZMINÆ SPERMOS INJEKCIJÀ) IR EMBRIONO PERKËLIMO PROCEDÛRÀ

S a n t r a u k a

Tikslas. Išsiaiškinti endometriumo storio ir tipo, moters amþiaus, kûno masës indekso (KMI), kiauðidþiø stimuliacijos protokolo ir embrionø ádëjimo á gimdà kokybës poveiká pastojimui atliekant IVF, ICSI.

Tyrimo metodai. Atliktas retrospektyvinis tyrimas. Iðnagrinëtos *252* moterø, gydytø dël nevaisingumo „Vaisingumo klinikoje" 2000–2003 m., ligos istorijos. Ðioms moterims taikytas dirbtinis apvaisinimas mëgintuvëlyje, áskaitant ir intracitoplazmines spermijø injekcijas, per 311 gydymo ciklø. Vertinta: 1) ultragarsinis endometriumo tipas, 2) endometriumo storis chorioninio gonadotropino injekcijos (toliau – hCG) dienà; 3) moterø amþius; 4) KMI; 5) kiauðidþiø stimuliacijos protokolas bei 6) embrionø ádëjimo á gimdà kokybë.

Rezultatai. Atlikus 311 dirbtinio apvaisinimo ciklø 252 moterims, pastojo 92 moterys, nëðtumo daþnis buvo 29,6%. I endometriumo tipo atveju nëðtumo daþnis buvo gerokai didesnis – 38,7%, lyginant su 7,8% pastojusiø moterø, kurioms buvo nustatytas II tipo endometriumas (p < 0,0001).

Moterø, jaunesniø nei 35-iø, nëðtumo daþnis buvo didesnis nei vyresniø ir atitinkamai siekë 36,1 ir 19,5% (p = 0,002). Lyginant ilgo, trumpo, „su antagonistais" kiauðidþiø stimuliavimo protokolø poveiká nëðtumo daþniui, nustatyta, kad statistiðkai patikimai palankesnis ilgas stimuliavimo protokolas (p = 0,028). 220 ciklø metu atlikus puikø embrionø ásodinimà, gauti 75 klinikiniai nëðtumai (nëðtumo daþnis – 34,1%). Didþiausià átakà klinikinio nëðtumo daþniui turëjo ásodinimo kokybë, lyginant puikià su prasta (OR-5,34, 95% CI 1,49–3,07). Statistiðkai patikimai daþniau pastojo moterys, kurioms buvo taikytas ilgas stimuliavimo protokolas lyginant su trumpu (OR 2,31, 95% CI 1,19–4,47). Kiekvienas papildomai ágimdà patalpintas embrionas galimybæ pastoti padidino 2,14 karto (95% CI 1,49–3,07).

Išvados. Nustatyta, kad statistiškai patikimai daþniau pastodavo moterys, kai: a) hCG injekcijos dienà buvo nustatytas I tipo endometriumas, b) hCG injekcijos dienà endometriumas buvo storesnis nei 11 mm, c) buvo naudojamas ilgasis kiauðidþiø stimuliacijos protokolas, d) pavykdavo uþtikrinti puikià embrionø ádëjimo á gimdà kokybæ. Taip pat patikimai pastojo jaunesnio amþiaus moterys, jei palyginsime jas su nepastojusiomis.

Raktaþodþiai: IVF, ICSI, endometriumas, prognostinë vertë