Pregnancy prediction models and eSET criteria for IVF patients—do we need more information?

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Abstract *Purpose:* The purpose of the present study was to evaluate statistical prediction models and simple allocation criteria, based on predictors for pregnancy, as tools to identify a good prognosis group in a possible eSET setting.

Methods: A pregnancy prediction model based on logistic regression models was generated by analysis of 1675 DET treatment cycles. The model was evaluated and compared to simple eSET allocation criteria.

Results: Embryo quality, patient age, and basal FSH were identified as significant predictors (at 5% significance level) of pregnancy. Although comparable to previously generated models, the predictive ability of the present model was relatively poor and practically similar to simple allocation criteria based on age and embryo quality.

Conclusions: Existing prediction models, or simple allocation criteria, are limited in identifying good prognosis patients. Future studies of the applicability of improved pregnancy prediction models will need very comprehensive and detailed patient and embryo information.

Keywords Single embryo transfer · Prediction models · Good prognosis group

Introduction

The adverse effects of multiple births associated with assisted reproductive technologies [e.g. 1, 2, 3, 4, 5, 6] have led to guidelines restricting the number of embryos to be transferred. Until now, two embryos have become standard in many European countries, and in particular in the Nordic countries. A study from 1998 demonstrated that the chance of birth was not diminished by transferring two embryos as compared to the transfer of three or more embryos [7]. However, even with a restriction of two embryos per transfer, the probability of twin pregnancy today remains high at around 25% [8]. Elective single embryo transfer (eSET) to selected women with a high probability of multiple pregnancies comprises an increasing proportion of assisted reproduction cycles, primarily in Sweden and Finland. The clinical challenge in eSET is to create a balance between maximizing the opportunity for the infertile couple to have a child on the one hand and the need to minimize the risk of multiple pregnancies with subsequent risk of harm to the future child and family on the other.

Elective SET may result in a limited decrease in “baby take home” rate among selected patient groups [9, 10], especially if an effective cryopreservation program is operative [11]. The choice of eSET versus double embryo transfer (DET) can be highly sensitive to individual preferences, and it has been discussed how to allocate couples to eSET based upon evidence based principles [12]. Therefore, objective statistical models for predicting probability of pregnancy have been suggested as tools to identify candidates for eSET, i.e. patients with a high probability of pregnancy [13–16].

The aim of the present study was to identify predictors of pregnancy, and in a statistical model evaluate the predictive power of a model based on variables recorded in a fertility

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database covering all treatment cycles in a large, public fertility clinic. Elective SET is primarily an option in fresh cycles; hence the model was based on information from fresh cycles. Studies of elective single embryo transfer have used the woman's age and embryo quality (morphology and developmental stage) as parameters for allocation to eSET [9, 10, 11, 17, 18, 19, 20, 21]. The predictive power of the model generated in this study was compared to such rather simple guidelines. Finally, the general ability to identify good prognosis patients, whether using prediction models or simple guidelines, is discussed. In the context of this work, a predictor is understood as any recorded piece of information that supports a probabilistic estimate of a pregnancy within a treatment cycle.

Materials and methods

Study population

We used a database containing conventional clinical and laboratory information on IVF and ICSI treatment cycles performed at a public fertility clinic from 2000 through 2003. National regulations ensuring anonymity and privacy with respect to patient data were followed. The mean age of the women was 31.9 years (SD 4.03, range 19–42). Indications for treatment were: Male factor (42.7%), idiopathic (26.2%), tuba factor (23.4%), and endometriosis (7.8%). The clinical pregnancy rate among the 2193 DET cycles was 35.6% (759/2194), and the twin pregnancy rate was 31.5% (239/759) of pregnancies, and 10.9% (239/2194) of all treatment cycles. Cycles with frozen embryo transfer,

SET transfers, and cycles not resulting in ET were excluded from the analysis (Fig. 1). Hence, only DET cycles were included.

Treatment protocol

In the large majority of cycles long down regulation was performed from cycle day 21 with Buserelin[®] nasal spray 0.1 mg six times per 24 hours. Following a menstrual bleeding, stimulation was started two weeks later with a starting dose of 100 or 150 IU Puregon[®] per day, individually adjusted. First follicular ultrasound was done on the 10th stimulation day. In a minority of cycles either clomiphene citrate (Pergotime[®]) alone (100 mg per day from cycle day 3 to seven) or in combination with HMG (Menopur[®]) 150 IU from day 7 was used. In the latter case an antagonist (Cetrotide[®] 0.25 mg per day) was added when the leading follicle was ≥ 14 mm. When 3–4 follicles of ≥ 18 mm were present HCG (Profasi[®] or Pregnyl[®], 10,000 IU) was given 37 hours before oocyte pick-up (OPU). For OPU, patients were given i.v. Phentanyl 0.25 mg, and a paracervical block with lidocaine. OPU was performed ultrasound-guided transvaginally with a double-lumen needle (Cook[®], 16G) in oocyte collection media (Medicult[®]) with flushing of each follicle as necessary. Luteal phase support was given with progesterone vaginally (Progestan[®], 100 mg $\times$ 4) and continued in case of a positive pregnancy test for an additional three weeks.

Measurement of serum HCG was performed two weeks following embryo transfer. A level of ≥ 20 IU was considered a positive test. Pregnant patients were scanned routinely 5 weeks after embryo transfer. Clinical pregnancy

Fig. 1 Diagram showing the data selection procedure. *FER: Frozen embryo replacement †SET: Single embryo transfer ‡DET: Double embryo transfer

was defined as a live intrauterine pregnancy at this scan. The implantation rate was defined as the number of gestational sacs divided by the number of embryos transferred.

Laboratory procedures

Oocyte handling and ICSI procedure was performed as previously described [22]. During the study period, two embryos were transferred routinely. Single embryo transfer (SET) was performed when only one transferable embryo was present, in case of patient private preferences, or in case of clear medical contraindications for twin pregnancy (e.g. cervical incompetence, heart disease etc.). In vitro culture was ended on day two after oocytes pick up and fertilisation, where all embryos were transferred, frozen or discarded.

Scoring of embryo quality

Embryo scores were recorded in the database according to developmental stage and morphology [23] prior to transfer on day 2 after aspiration and fertilisation. Embryo quality was scored 1.0 if the embryo had blastomeres of equal size without fragmentation, 2.0 in case of unequal blastomeres without fragmentation, 2.1 if fragmentation was < 10%, 2.2 with 10–19% fragmentation, 3.1 with 20–49% fragmentation, and 3.2 with > 50% fragmentation. An embryo was considered transferable if fragmentation was < 50%, and if at least 2 cells were present on day 2. The two highest scoring embryos were transferred. A combined numeric score for the developmental stage and the morphology was generated for the prediction model according to Table 1.

Statistical analysis

All fresh, two-embryo transfer cycles were selected for analysis ($n = 2193$). Predictor candidates were chosen if they were known to be important predictors of treatment result, or if there was a theoretically good reason for inclusion [24,

25, 14, 13]. The following candidates were included: The woman's age, duration of infertility, body mass index (BMI, calculated from patient self-reported height and weight), basal FSH on cycle day 3–5, treatment type (IVF or ICSI), indication for treatment, number of oocytes retrieved, fertilisation rate, numeric score of best embryo, numeric score of second best embryo. Continuous variables that were not linearly associated with the outcome (e.g. age, see Table 2) were categorized. Odds ratios were calculated for each group to identify possible non linearity in odds ratio change between groups within the same variable. Details are given in Table 2.

First we performed univariate logistic regression analyses, including the variables one at a time. We subsequently included potential predictor candidates from the univariate analysis and performed stepwise forward and backward logistic regression to estimate the best possible multivariate prediction model. However, the forward and backward elimination procedure generated identical models, and hence only one model (backward elimination) is presented. Results from the logistic regression of the individual variables are presented as odds ratios (OR) with 95% confidence interval (CI). The multivariate model is presented with variable coefficients. Variables were included in the model at $P < 0.1$. We also calculated sensitivity, specificity, and positive and negative predictive values for the model. Because the different treatment cycles from the same patient cannot be considered independent observations, robust variance estimates taking into account this non-independence were obtained.

All statistical analyses were performed using the STATA software (Stata intercooled version 8.2 for windows, www.stata.com).

Results

Univariate analysis of the association between predictors and pregnancy

The unadjusted associations between predictor candidates and the probability of pregnancy (1 or 2 live fetuses) and twin pregnancy (2 live fetuses) for all DET treatment cycles are shown in Table 1.

Based on the initial analysis and previous studies, the following predictor candidates were chosen for possible inclusion in a pregnancy prediction model based on all two embryo transfer cycles: Age (five categories), BMI (four categories), basal FSH on menstrual cycle day 3–5 (two categories), treatment indication (four categories), number of oocytes retrieved (three categories), fertilisation rate (six categories), numeric score of best and second best embryos transferred.

Table 1 Assignment of numeric scores according to cell number and fragmentation day 2 following insemination

Cell number	Morphology score	Combined numeric score (low number = best quality)
4	<=2.2 (10–20% fragments)	1
Not 4	<=2.2 (10–20% fragments)	2
4	=3.1 (20–50% fragments)	2
Not 4	=3.1 (20–50% fragments)	3

Table 2 Association between predictor candidates and probability of pregnancy and twin pregnancy given by odds ratios (OR) with 95% confidence interval (95% CI)

Independent variable	Groups	OR (95% CI)	
		Pregnancy model	Twin pregnancy model
Age, each year	Year (<i>n</i> = 2193)	0.94 (0.92–0.96)	0.91(0.88–0.94)
Age group	1: 19–24 (<i>n</i> = 71)	0.67 (0.40–1.13)	0.55 (0.25–1.25)
	2: 25–29 (<i>n</i> = 551)	1	1
	3: 30–34 (<i>n</i> = 942)	0.78 (0.63–0.97)	0.67 (0.50–0.90)
	4: 35–39 (<i>n</i> = 586)	0.53 (0.41–0.68)	0.30 (0.20–0.46)
	5: 40+ (<i>n</i> = 43)	0.32 (0.15–0.70)	–
Duration of infertility (months)	1: 0–12 (<i>n</i> = 117)	1	1
	2: 13–24 (<i>n</i> = 489)	0.83 (0.55–1.27)	0.59 (0.33–1.04)
	3: 25–36 (<i>n</i> = 518)	0.81 (0.54–1.23)	0.70 (0.40–1.22)
	4: 37–48 (<i>n</i> = 349)	0.77 (0.50–1.18)	0.71 (0.39–1.27)
	5: 49–60 (<i>n</i> = 189)	0.79 (0.49–1.27)	0.54 (0.27–1.08)
BMI	6: > 60 (<i>n</i> = 531)	0.82 (0.55–1.25)	0.54 (0.30–0.95)
	1: < 18.5 (<i>n</i> = 57)	1.24 (0.72–2.12)	1.57 (0.77–3.17)
	2: 18.5–25 (<i>n</i> = 1306)	1	1
	3: > 25–30 (<i>n</i> = 414)	0.81 (0.64–1.02)	0.83 (0.58–1.19)
	4: > 30 (<i>n</i> = 189)	0.83 (0.60–1.15)	0.92 (0.57–1.50)
Basal FSH on menstrual cycle day 3–5	missing (<i>n</i> = 227)*		
	1: 0–10 IU (<i>n</i> = 1733)	1	1
	2: > 10 (<i>n</i> = 114)	0.56 (0.36–0.88)	0.29 (0.10–0.78)
Treatment type	Missing (<i>n</i> = 346)*		
	1: IVF (<i>n</i> = 1356)	1	1
Indication for treatment	2: ICSI (<i>n</i> = 837)	0.95 (79–1.14)	1.08 (0.82–1.43)
	1 Idiopathic (<i>n</i> = 574)	1	1
Number oocytes retrieved	2 Male factor (<i>n</i> = 936)	0.98 (0.79–1.22)	1.11 (0.79–1.55)
	3 Tuba factor (<i>n</i> = 513)	0.84 (0.65–1.08)	1.03 (0.70–1.51)
	4 Endometriosis (<i>n</i> = 170)	0.85 (0.59–1.22)	0.81 (0.45–1.47)
	2: 1–5 (<i>n</i> = 697)	1	1
Fertilisation rate %	3: 6–10 (<i>n</i> = 959)	1.20 (0.97–1.47)	1.20 (0.88–1.65)
	4: 11+ (<i>n</i> = 537)	1.16 (0.91–1.47)	1.14 (0.79–1.65)
	1: 0 (<i>n</i> = 0)		
	2: > 0–20 (<i>n</i> = 38)	1.11 (0.89–1.39)	0.68 (0.21–2.27)
Score of best embryo	3: > 20–40 (<i>n</i> = 245)	0.94 (0.69–1.28)	0.79 (0.48–1.29)
	4: > 40–60 (<i>n</i> = 512)	1.00 (0.79–1.27)	1.00 (0.70–1.43)
	5: > 60–80 (<i>n</i> = 675)	1.11 (0.89–1.39)	1.06 (0.76–1.47)
	6: > 80–100 (<i>n</i> = 718)	1	1
Score of second best embryo	Missing (<i>n</i> = 5)*		
	1 (<i>n</i> = 1356)	1	1
	2 (<i>n</i> = 745)	0.47 (0.38–0.57)	0.41 (0.29–0.57)
Missing (<i>n</i> = 4)*	3 (<i>n</i> = 120)	0.35 (0.22–0.56)	0.26 (0.11–0.65)
	1 (<i>n</i> = 468)	1	1
	2 (<i>n</i> = 1079)	0.71 (0.57–0.88)	0.56 (0.41–0.76)
	3 (<i>n</i> = 670)	0.38 (0.29–0.49)	0.28 (0.19–0.42)

*Missing values not included in the analyses.

Pregnancy prediction model, multivariate analysis

Results from multivariate analyses stratified by cycle number did not support the hypothesis that variables in the model would predict pregnancy in a substantially different manner in the different treatment cycles outlined in Fig. 1 (results

not shown). A multivariate analysis stratified by age was also conducted. The stratified models were neither significantly nor substantially different. A combined model for all treatment cycles and age groups was therefore generated, with treatment cycle number and age group included as independent variables. Of the 2193 eligible cycles, 518 had one or

Table 3 (A) All treatment cycles with two embryos transferred (pregnant vs. not pregnant). Standard errors adjusted for clustering on individual patient; (B) All treatment cycles with two embryos transferred (twin pregnant vs. singleton or not pregnant). Standard errors adjusted for clustering on individual patient

	Coefficient	Std. Err	Z (Coeff / std. err.)	<i>P</i> > Z	CI (95%)
A					
Agegrp	− 0.295	0.065	− 4.54	0.000	− 0.422; − 0.167
BMI 4group	− 0.131	0.075	− 1.74	0.082	− 0.278; 0.016
Basal FSH	− 0.589	0.261	− 2.26	0.024	− 1.100; − 0.078
Score best embryo	− 0.505	0.115	− 4.37	0.000	− 0.731; − 0.278
Score Secondbest	− 0.251	0.089	− 2.83	0.005	− 0.425; − 0.076
Constant	2.417	0.443	5.46	0.000	1.549; 3.286
B					
Agegrp	− 0.442	0.091	− 4.87	0.000	− 0.619; − 0.264
BMI 4group	− 0.093	0.114	− 0.82	0.414	− 0.318; 0.131
Basal FSH	− 1.313	0.597	− 2.20	0.028	− 2.484; − 0.143
Score best embryo	− 0.534	0.197	− 2.71	0.007	− 0.920; − 0.169
Score secondbest	− 0.423	0.130	− 3.26	0.001	− 0.678; − 0.169
Constant	2.364	0.786	3.01	0.003	0.824; 3.905

more missing observations among the included variables. Hence, 1675 cycles were included in the analyses (579 first cycles, 497 second cycles, and 599 third or higher number cycles) Table 3.

Comparison of the coefficients for the pregnancy (twin or singleton vs. not pregnant) and the twin pregnancy models, supports the hypothesis that prediction of twin pregnancy is based on the same variables and coefficients as the prediction of pregnancy (twin plus singleton). As expected, the coefficient for second best embryo score was slightly higher for the twin pregnancy model, although not significantly so.

Table 4 is a classification table for the model. Coefficients from the combined pregnancy model were used to calculate a probability of pregnancy for each patient from the eligible cycles. Patients were then classified into two groups: Predicted pregnant if the probability *P* of pregnancy *P*(pregnancy) was calculated to be ≥ 0.3 and predicted not pregnant if *P* (pregnancy) < 0.3 (cut off value = 0.3).

The sensitivity of the model (using a cut off value = 0.3) was $(467/585) = 0.80$, which is the proportion of women predicted pregnant by the model among those who actually achieved a pregnancy. The specificity of the model was $(439/1090) = 0.40$, which is the proportion of women predicted not pregnant by the model among those who did not achieve a pregnancy. Using a cut off value of 0.3, the pos-

itive predictive value was 41.8%. That is, given a patient was predicted pregnant, her probability of achieving pregnancy was 41.8%; and the negative predictive value was 78.8%, i.e. given a patient was predicted not pregnant by the model, her probability of not achieving pregnancy was 78.8%.

For a patient with at least two transferable embryos after a successful stimulation, oocyte pick up, and fertilisation (e.g an eSET candidate) the prior probability of achieving pregnancy, among all the 1675 cycles included in the regression model, was $(584/1675)$ 34.5%. When the model was used to allocate such patients into two groups (high chance of pregnancy and low chance of pregnancy), the probability of pregnancy in the high chance group was calculated to be 41.8% at a cut-off *P* = 0.3. Probability raised to 45.2% at cut-off *P* = 0.36, but the group became substantially smaller when applying this cut off value. The negative predictive value increased from an a priori value of 65.5% to 78.8% in the low chance group (cut-off = 0.3) and 73.8% (cut-off = 0.36). Choice of cut-off probability level can be motivated according to allocation decisions. A cut-off value of 0.33 divides the patients in two approximately equally sized low and high chance groups. Table 5 shows positive and negative predicted prior and posterior probability of pregnancy at selected, different cut-off *P*-values. Because it is difficult for

Table 4 Classification table for the model

	True outcome, observations from year 2000–2003 treatment cycles		
	Actually pregnant	Actually not pregnant	Total
Predicted pregnant	467	651	1118
Predicted not pregnant	118	439	557
Total	585	1090	1675

Table 5 Positive and negative predicted prior and posterior probability of pregnancy at selected different cut-off P -values

	Cut-off $P = 0.3$		Cut-off $P = 0.36$		Cut-off $P = 0.46$	
	Positive predicted %	Negative predicted %	Positive predicted %	Negative predicted %	Positive predicted %	Negative predicted %
Pregnancy model						
Posterior probability	41.8	78.8	45.2	73.8	50.7	68.6
Prior probability all cycles	35.5	65.5	34.5	65.5	34.5	65.5
Twin preg. Model						
Posterior probability	16.7	88.5	20	88.7	–	88.5
Prior probability all cycles	11.4	88.6	11.4	88.6	11.4	88.6

the model to predict pregnancy with great accuracy nearly all patients will have a calculated probability of pregnancy between 25 and 50%. Therefore, cut off values outside this range will not be meaningful.

ROC-curves for the pregnancy model (area under curve = 0.64) and the twin pregnancy model (area under curve = 0.68), presents visually the relatively poor predictive ability of the models (Fig. 2).

The model was also evaluated on 180 cycles from 2004 (Table 6). These observations were not included in the dataset used for model generation. Positive (34%) and negative (66%) predictive values were expectedly lower than when the model was applied on the 2000–2003 cycles that were used to generate the model.

Discussion

In this study we identified predictors of pregnancy, and evaluated their predictive power. We presented a prediction model based on 1675 observations which included several significant predictors. One significant predictor, basal FSH, had for practical reasons been measured arbitrarily from cycle day 3–5. This variable could potentially predict pregnancy better if recorded more consistently on the same cycle day. BMI, calculated from patient self-reported height and weight

prior to the first treatment cycle, was a significant predictor only at a level of $P = 0.10$ level but not at $P = 0.05$. A controlled recording of patient weight just before a treatment cycle (and height at least once) could improve the quality of the BMI data and hence the predictive power of this variable. The same factors apparently influenced the chance of (any) pregnancy as well as multiple pregnancies, demonstrating the possibility of using one prediction model. This was also recognised by [14].

Comparison of the prediction model and simple eSET inclusion criteria: If the prediction model was used to identify good and bad prognosis groups, then the positive predictive value as well as the negative predictive values within the groups was only limitedly increased, when compared to random allocation of patients, with at least two transferable embryos. Yet the present model carries approximately the same predictive capacity as previous models published [13, 14]. Patient age and embryo score were also the most important predictors in these studies. Therefore, with the treatment information conventionally available about patients and embryos, statistically generated prediction models with several variables cannot be considered powerful tools when it comes to identifying patients with a particularly high probability of achieving pregnancy. Identification will, even with the best current prediction models available, carry a high proportion of randomness in relation to probability of achieving

Fig. 2 Receiver Operating Characteristic for the pregnancy prediction models. (ROC-curve); A: Pregnancy model (area under curve = 0.6421); B: Twin pregnancy model (area under curve = 0.6869)

Table 6 Classification table for some observations not included in model generation

	True outcome, observations from year 2004 treatment cycles		
	Actually pregnant	Actually not pregnant	Total
Predicted pregnant	51	95	146
Predicted not pregnant	10	24	34
Total	61	119	180

pregnancy, as age and embryo score are still relatively poor predictors for the individual patient. When the current model was applied on cycles not included in the model generation, but still from the same clinic, the predictive value of the model was further diminished, and it is not likely to improve when applied on data from other clinics. So far, the search for predictors among commonly registered variables in ART databases have not resulted in significant progress in the identification of strong predictors of pregnancy.

As the main predictors identified so far relate to female age and embryo quality (morphology and developmental stage) it is not surprising that simple guidelines for eSET restricted to the same parameters, typically recommending eSET if age < 37 and two top embryos available [26], seem to be comparable to advanced prediction models when it comes to identifying good prognosis patients. Typically, half or less of the total population of fresh cycles meet such criteria [11, 17, 19]. Thus approximately 20% of treatment cycles in the present database would have met the criteria. Retrospective analysis of 2193 fresh cycles in the present database, with two transferable embryos, shows that the pregnancy rate was 48% in the cycles meeting eSET criteria (approx 20% of fresh cycles), compared to 32% in treatment cycles not meeting eSET criteria (approx 80% of fresh cycles), so even in rigorously selected fresh transfer cycles (403/2193) the risk of treatment failure was 52%, compared to 65% looking at all 2193 cycles.

We therefore argue that, based on information conventionally recorded in fertility databases (including the present) it is difficult to separate patients in good and bad prognosis groups, whether using prediction models or simple allocation criteria. More information is needed. We suggest that more detailed and higher quality information on existing parameters (e.g. embryo morphology, patient characteristics) in combination with new predictor candidates, may answer the important questions which patients will become pregnant, but this will have to await the availability of such an elaborate database. Practitioners of ART definitely need more information to improve the identification of good prognosis patients. This present difficulty in predicting pregnancy among ART patients is widely recognised, and the search for new and improved predictors is ongoing, including improved morphology scoring, spindle visualization imaged by Polscope [27], better embryo scoring and in particular chromosomal

characteristics such as aneuploidies [28, 29, 30, 31]. Physiological measures such as amino acid turnover [32, 33], oxygen consumption [34, 35], presence of embryonic HLA-G [36], and embryo production of platelet activating factor [37] could be relevant. Also, more detailed patient characteristics could improve the ability to predict pregnancy among ART patients and improve treatment success rate while reducing frequency of multiple births. Biochemical and genetic markers of endometrial receptiveness has been suggested in a review [38], together with physiological markers such as utero-ovarian vascular impedance [39]. Life style factors, which are known to increase time to pregnancy among naturally conceiving couples [40], could also prove to be potential predictor candidates.

It is interesting that in Sweden, SET represented 67% of all transfers in 2004, with an unchanged delivery rate of 27% per transfer while the IVF multiple birth rate was reduced to 5.6% [41]. However, it cannot be excluded that in case of an unchanged DET policy the clinical pregnancy rate may have increased.

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