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The Mathematical Outcome of The IVF, Including Early Cleavage

IVF Sonuçlarının, Erken Klivaj'ı da İçeren Matematiksel Sonuçları

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Abstract

Aim: In this manuscript, the examination of independent factors for the success of IVF (In Vitro Fertilization) was aimed to be conducted.

Material and Methods: Hormones, including Anti-Mullerian Hormone (AMH), Follicle Stimulating Hormone(FSH), the growth stages of the oocyte that turns into an embryo (total fertilized oocyte, early cleavage, etc.), the medical adjustments were included. Our dependent factor, the success of fertilization, was categorized into two groups; in other words, binary logistic regression for pregnancy success was examined.

Results: For the statistical significance of our study, Omnibus Tests of Model Coefficients (p-value=0,008) and Hosmer and Lemeshow tests (p-value=0,462) were conducted. Our model was successful through statistical meaningfulness. With a Chi-Square test with a p-value of close to zero, women's age groups were proven different for groups below and over the age of 35 for InVitro Fertilization (IVF)

Discussion and conclusion: Study shows Early Segmentation increases the chances of pregnancy; In contrast, the woman's age and FSH reduce the likelihood of pregnancy.

Keywords: IVF, FSH, early cleavage, binary logistic regression, odds values.

Öz

Amaç: Bu araştırmada IVF (In Vitro Fertilizasyon) başarısı için bağımsız faktörlerin incelenmesi amaçlanmıştır.

Gereç ve Yöntem: Anti-Mullerian Hormon (AMH), Folikül Uyarıcı Hormon (FSH) gibi hormonlar, embriyoya dönüşen oositin büyüme aşamaları (total döllenmiş oosit, erken bölünme vb.), tıbbi ayarlamalar dahil edildi. Bağımlı faktörümüz olan döllenme başarısı ve gebelik iki gruba ayrıldı; diğer bir deyişle gebelik başarısı için ikili lojistik regresyon incelendi.

Bulgular: Çalışmamızın istatistiksel anlamlılığı için Model Katsayılarının Omnibus Testleri (p-değeri=0,008) ve Hosmer ve Lemeshow testleri (p-değeri=0,462) yapıldı. Modelimiz istatistiksel anlamlılık yoluyla başarılı oldu.P değeri sıfıra yakın Ki-Kare testi ile Tüp Bebek (Tüp Bebek) için 35 yaş altı ve üstü kadınların yaş gruplarına göre farklı olduğu kanıtlandı.

Tartışma ve Sonuç: Çalışmamız sonuçlarına göre , erken segmentasyon gebelik şansını artırmakta; buna karşılık kadının yaşı ve bazal serum FSH düzeyi gebelik olasılığını azaltmaktadır.

Anahtar Kelimeler: IVF, FSH, erken bölünme, ikili lojistik regresyon, olasılık değerleri.

INTRODUCTION

The importance of the age of the recipients and the FSH levels has been widely known during the years of the IVF process (1). In order to reduce multiple birth rates without reducing overall birth rates, it has been shown that human embryos that complete the first mitotic division within 25-27 hours after fertilization in the process of selecting the most suitable embryos for transfer yield higher pregnancy and implantation rates (2). The method of transferring blastocysts has also brought to attention the critical role of the 2-blastomere stage in the early hours of the process. Our research has studied this fact with the predictor "Early Cleavage" . In 2023, Van Marion et al. (2023) wanted to develop a TLM prediction model that could predict pregnancy chances, including early cleavage data, after both single and double embryo transfer (SET and DET) (3). The researchers' work demonstrated that the application of the EU statistical model is a novel approach to develop a TLM prediction model. The researchers suggested in this study that it could aid in embryo selection and decision making for SET or DET, with further development and validation in clinical practice (3).

R.A. Fisher's revolutionary studies in 1908 and 1915 intro-

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duced the idea of Degrees of Freedom and the distribution of correlation coefficients (4). In assessing the Distribution of Degrees of Freedom and Correlation Coefficients, Guyatt and colleagues examined known problems and combinations of observations to generalize least squares theory; the number of observations and the unknown factors included serve as divisors (5,6). These articles were used to estimate the Standard Errors of the datasets in our study (1).

Evaluations on IVF, Dodson et al. it is one of the first studies conducted in 1957 (6). Both of these research areas are explored in the same article: Duran et al. In 1998 he published an article on both, including IVF and logistic regression (7).

Cai et al. investigated a possible positive correlation between GDM (gestational diabetes) and IVF risk factors of Body Mass Index (BMI)(8).

In our study, we investigated the hormones that are effective in the IVF process, including antimullerian hormone (AMH) and follicle stimulating hormone (FSH). Lekamge et al. explains the difference between low and high AMH levels below 8 pmol/l (9). Thus, they found a positive correlation between the two predictors.

And oocytes-specific factors include antral follicle count (AFC), early division (EC), and embryo development stage. The early cleavage effect of the oocyte has an important place in our study; The results reported by Bos-Mikich et al. are as we have found, as previously reported in various studies; indicates that early division of oocytes increases pregnancy rates (10). In our study, we aimed to develop a mathematical model using these parameters.

MATERIAL and METHOD

The data in this study consists of 458 patients followed up except for Tese/microtese cases who received ART treatment in Istanbul University Cerrahpaşa Medical Faculty Hospital IVF Laboratory between October 2010 and December 2012. In addition to the development data of the embryos of these cases, basal FSH, LH, E2, PRL values, AMH and TSH values on the 3rd day of menstruation, E2 and LH values on the day of HCG injection, oocyte count, 2PN number, sperm parameters of the spouses and pregnancy rates were analyzed. Azoospermic cases diagnosed with male infertility were excluded from the study.

Ovarian Stimulation

For ovarian stimulation, from the second day of menstruation, 150-225 IU of follicle-stimulating hormone (rFSH; Gonal-F®; Merck Serono, Turkey) was applied according to the age and weight of the woman. When the primary follicle reached a 12-13 mm diameter, 0.25 mg daily of gonadotropin-releasing hormone (GnRH) antagonist (Cetrotide®; Merck Serono, Turkey) was started. Follicle maturation was achieved with 250 μg of recombinant human chorionic gonadotropin (r-hCG; Ovitrelle®; Merck Serono, Switzerland) when two or more follicles reached 18 mm in diameter. OPU was performed 36 hours after injection. To support the luteal phase after ET, 100 mg of progesterone (Progynex™; Farmako-Koçak, Turkey) was administered intramuscularly for 12 days. Intravaginal progesterone gel was applied 12 days after implantation (Crinone® 8%; Merck Serono, Switzerland). Serum β-hCG was measured 14 and 16 days after the OPU procedure, and early pregnancy was monitored by

Table 1: Means and standard deviations in the study group.

	n	Mean	Std. Deviation	Minimum	Maximum
AMH	314	3,42	2,78	0,01	16,70
FSH	410	6,72	3,26	0,10	28,30
Total oocyte	424	8,46	5,25	1,00	34,00
Polar body (normal)	415	5,71	4,05	0,00	23,00
ZP (normal)	415	6,23	4,26	0,00	30,00
Oocyte dimention (normal)	415	6,15	4,03	0,00	23,00
Total fertilization oocyte	410	3,93	3,02	0,00	20,00
Blastomere (2nd day-2)	256	2,07	1,40	0,00	9,00

Table 2: Age and early cleavage frequencies

		Frequency	Percent
Age	<=35	362	82,3
	>35	78	17,7
	Mean ± Std. Deviation	31,81 ± 4,91 (440)	
Early cleavage (n=385)	No	304	79,0
	Yes	81	21,0

transvaginal ultrasound at the seventh week. Clinical pregnancy was defined by fetal heartbeat seen on transvaginal ultrasound. Progesterone treatment was continued until the tenth week of pregnancy.

With Binary Logistic Regression, the patients' state of pregnancy were divided into two groups; pregnant is shown by 1 and non-pregnant by 0.

Basal hormone values (AMH, LH, FSH, HCG included) and fertilized oocyte through the stages of blastomere (varying from 2-cell-blastomere to 8 cells) to the transferred embryo (from the first hours to the 4th day) were examined. Embryos' development includes the early stages; oocytes' condition through the stages of fertilization, including the GV (Germinal Vesicle), Metaphases 1 and 2 and degenerated oocytes also take place in the study.

Later forms Transferred Embryo are examined as; Morula, Compact morula and Blastokist and finally pregnancy.

Moreover, The main components of the fertilizated oocyte have an important place in the outcome. From this aspect we see the the quality of these components; oocyte granulation, refractery body(RB), cytoplasm thickness , polar body (PB) fragmentation and sizes, zona pellucida (ZP) thickness and the size of oocytes' are also used to form a mathematical formula using Binary Logistic Regression.

Finally our dependent Pregnancy was turned into a formula starting from the oocyte.

Statistical Analysis

The results are obtained using SPSS's PASW Statistics Version 18. The effect of Age, AMH, FSH, Total oocyte, Polar body (normal), ZP (normal), Oocyte dimention (normal), Total fertilized oocyte, Blastomere (2nd day-2) and early cleavage variables on conception status Logistic Regression (stepwise) were analyzed by analysis. Logistic Regression Analysis was applied because the conception status is a binary variable. The analysis modeled age, early cleavage and

Table 3: Omnibus Tests of Model Coefficients

		Chi-square	df	Sig.
Step 1	Step	24,007	10	0,008
	Block	24,007	10	0,008
	Model	24,007	10	0,008

H_0: The model is not significantly effective for the combined effect of factors on the outcome variable.
H_1: The model is significantly effective for the combined effect of factors on the outcome variable.

Table 4: Hosmer and Lemeshow Test

Step	Chi-square	df	Sig.
1	7,714	8	0,462

FSH variables in step 3; left other variables out of the model.

The Logit Conversion

As neither the extremes do not follow a linear trend nor the errors are normally distributed in constant across the entire range of data, applying the logit conversion to the dependent variable by using logistic regression solves these problems that exist in linear regression. The simple formulation of logit conversion for this method (logistic regression) is as follows:

logit(Y)=natural log (odds)=In $\pi/(1-\pi)=\alpha+\beta\chi$

π=Probability(Y = outcome of interest |

X = x,a specific value of X)=e^($\alpha+\beta\chi$)/(1+e^($\alpha+\beta$x))

where π is the probability of the outcome of interest or "event," such as FSH value , α is the Y intercept, β is the regression coefficient, and e = 2.71828 is the base of the system of natural logarithms (11,12).

RESULTS

Table 1 demographic values of the study group shows. Table 2 includes age and early cleavage frequencies.

The probability or Odds of the combination of the independent variables were checked for statistical effectiveness by the Omnibus Test of Models' Coefficients (p-value=0,008) and the Hosmer and Lemeshow Test (p-value=0,462). The results of both tests show our model is statistically effective (Table 3 and Table 4).

Because we have come to the block with the predictor variables in one step from the beginning in our stepwise model, we reach three same values; including the Block 1 model with the p-value of 0,008. So we reject the null hypothesis for 0,05 significance level.

The Hosmer and Lemeshow Test is the commonly used measure of goodness-of-fit and therefore is a chi-square based test. Our model, with the p-value=0,462. Finally, we can state that the result of this test shows good fit.

AMH, Total oocyte, Polar body (normal), ZP (normal), Oocyte dimension (normal), and Total fertilized oocyte averages for the young group are high and the FSH average is low. It is statistically significant (p<0.01)(Figure 1).

Total oocyte, Polar body (normal), BP (normal), Oocyte size (normal), Total fertilized oocyte averages are high in early cleavage embryos. It is statistically significant (p<0.01) (Figure 2).

AMH, Total oocyte, Polar body (normal), ZP (normal), Oocyte diameter (normal), Total fertilized oocyte averages of the pregnant group were high, and the FSH average was low. It is statistically significant (p<0.01)(Figure 3).

Those younger than 36 are 6.14 times more likely to become pregnant than older women. Early cleavage status is 3.02 times more likely to conceive; A decrease in FSH value by one unit increases this probability by 1.24 times (Table 5). Table 6 shows the evaluation of the age factor with the chi square test.

Table 7 shows the variables that determine the probability of pregnancy (negative/positive).

The predictor variables FSH (Follicule Stimulating Hormone) and Women age effect the outcome (pregnancy) negatively with the small p-values (p=0,027 and p=0,032 respectively.) and negative B values.

Whereas, EC (early cleavage) changes the same way with pregnancy with the p-value of 0,28.

Table 5: Variables affecting the conception process (Logistic Regression)

	B	S.E.	Wald	df	Sig.	Odds Ratio (OR)	95% CI.for OR	
							Lower	Upper
Age <=35 - >35)	1,816	0,771	5,546	1	0,019	**6,149**	1,356	27,879
Early cleavage (No - Yes)	1,107	0,484	5,227	1	0,022	**3,024**	1,171	7,808
FSH	0,216	0,107	4,082	1	0,043	**1,241**	1,006	1,530

Table 6: Chi-Square Test, Age factor in IVF.

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	14,295[a]	1	0,000		
Continuity Correction[b]	13,34	1	0,000		
Likelihood Ratio	16,116	1	0,000		
Fisher's Exact Test				0,000	0,000
Linear-by-Linear Association	14,264	1	0,000		
N of Valid Cases	458				

a : 0 cells (,0%) have expected count less than 5. The minimum expected count is 26,72.

b: Computed only for a 2x2 table.

H_0: Women at and below the age of 35 do not show a statistically effective difference with the women over the age of 35.
H_1: Women at and below the age of 35 show a statistically effective difference with the women over the age of 35.
As the p-value is below 0,05; the null hypothesis (H_0) is rejected. There is a significant difference between women with ages at and below age of 35 and over the age of 35 by the means of pregnancy with IVF.

DISCUSSION

According to the present studies, one of the reasons that higher FSH levels occur is younger age. Interestingly, sufficient FSH values that take place lead to younger women having increased quality oocytes and implanting after fertilization. Therefore, the age of women in the medical treatment of IVF and embryo parameters are two of the few critical reasons for IVF success (13).

In women, the Luteinizing hormone rises after the basal follicle number occurs. In the ovulation circle, rising serum FSH during the early menstrual phase leads to this result (14). Age also plays an essential role in this circle; FSH rises several years before LH and more than a decade before menopause (15).

Another study evaluated IVF stimulation parameters and found more than 30 predictors of live births (16). Among these predictors, the rate of blastocyst development, the total amount of gonadotropins administered, and the number of eight-cell embryos were most correlated with live births. As expected, the diminished ovarian reserve was negatively associated with live births.

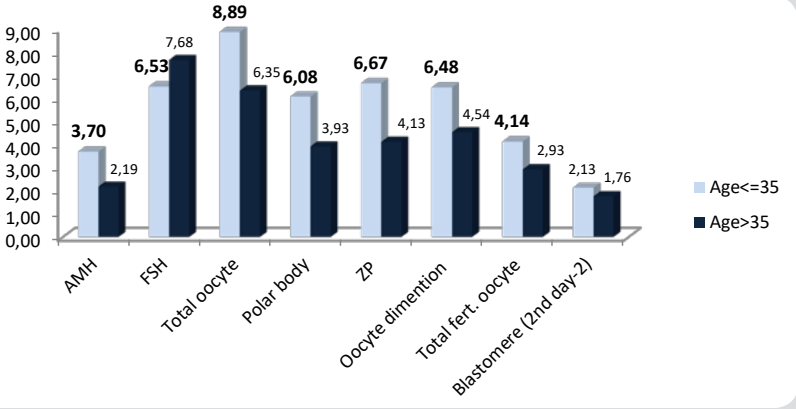

Figure 1: Averages and comparison according to age groups (Independent samples t Test). (Bold means are values that are statistically different.)

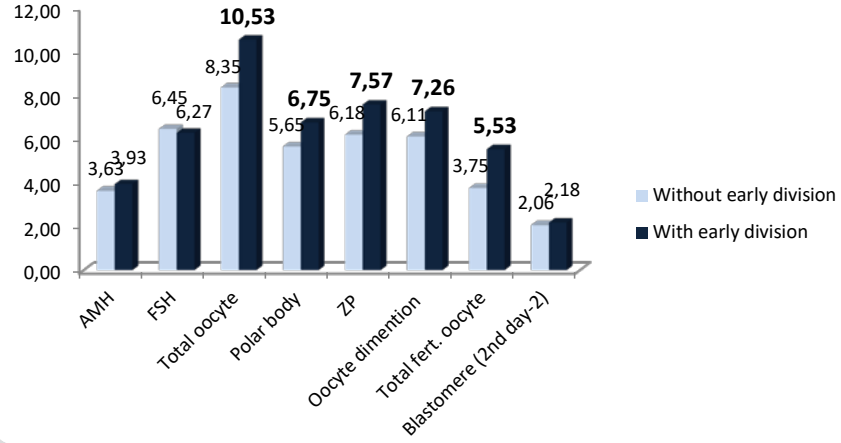

Figure 2: Means and comparison according to patient groups with or without early division (Independent samples t-Test). (Bold means are values that are statistically different.)

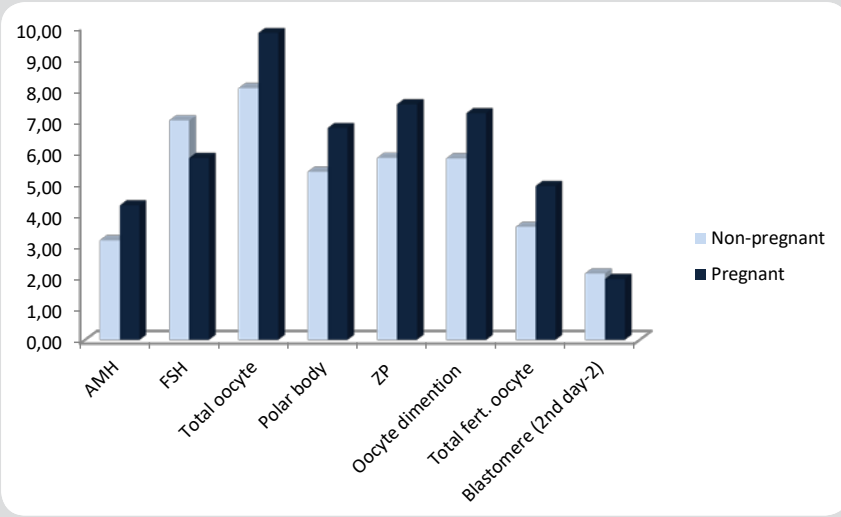

Figure 3: Means and comparison according to pregnant and non-pregnant patient groups (Independent samples t Test). (Bold means are values that are statistically different.)

Table 7: Predictors' ratios with pregnancy

Inverse ratio	Right ratio
Women age	AMH
FSH	Polarbody normal
Total oocyte	Total fertilizated oocyte
ZP normal	Early cleavage
Oocyte dimention normal	
2nd day 2 blastomere	

Our study also proves that early cleavage indicates a higher chance of pregnancy and is a strong indicator of embryo competence. Therefore, two blastomeres on the 25th to 27th hour should be considered a sign of a successful IVF process,.; whereas only two blastomeres on the second-day point to a lower possibility of pregnancy, as we see the result in our study. This fact can also be seen in the studies of Fenwick et al. (17).

The recent studies vary from the importance of fibroids to the application on women with blastocyst transfer with randomized trials (18,19,20).

In this study, we included the factors predicting IVF success; for example, ovarian reserve and FSH levels may be low in

older women (21,22). This can vary depending on the two factors we're talking about here. False hopes for pregnancy are a critical process from start to finish.

The determinants that may affect women with high FSH levels for a live birth should be thoroughly studied; Counseling is an option that can help. Counseling should include additional information about the IVF process and important determining variables. In addition, donor-oocyte or adoption options or need should be considered.

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