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Predictive Factors for Three Pronuclear Zygote in IVF Cycle

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Abstract

Introduction. Preimplantation Genetic Testing is one of the methods to screen genetic defects in embryos created through In Vitro Fertilization. In developing country this technique is still new and expensive method. Thus, knowing several factors in predicting the occurrence of three-pronuclear zygote can help much. **Method.** 472 cycles in 449 subjects who underwent controlled ovarian hyperstimulation in IVF cycles between January 2013 and August 2014 were included in the study. Categorical variables were compared using Chi Square test and continuous variables were analyzed using Independent t-test, and statistically significant was considered when $p < 0.05$. Multivariate logistic regression analysis was performed in order to correlate clinical variables and the occurrence of three-pronuclear zygote (3pn). **Results.** There were 38 3pn was identified in this study (8.05%). No correlation was found between age of the women, number of mature follicles, which are less than six with the incidence of 3pn. However, level of Anti Mullerian Hormones (AMH) found to be the strongest predictor with the incidence of 3PN ($p < 0.01$, RR 2.5, CI95% 1,55; 4.16). **Conclusion.** level of AMH is known to be strong predictor of thee pronuclear zygote after IVF cycle.

Keywords: Preimplantation genetic testing, IVF, three pronuclear, zygote, Anti-Mullerian Hormones

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1. Introduction

A common definition of infertility has been used in clinical settings is: "One year of unwanted non-conception with unprotected intercourse in the fertile phase of the menstrual cycles [1]."

Based on WHO, there are around 186 million married woman with infertility in developing country who is either having primary infertility or secondary infertility [2]. While in Indonesia, Yasmin Clinic that is located in Cipto Mangunkusumo General Hospital, Jakartam showing increasing of couple visit to get management of infertility from 1238 couples in 20013 to 1834 couples in 2014.

In Vitro Fertilization (IVF) becomes one of alternative method used in order to conceived. Pregnancy rate in Yasmin clinic Kencana is around 40 – 45% from all of the couple underwent IVF. In order to undergo IVF cycles, several factors contributes to the successfulness of it, for example the age of the patient, quality of endometrium, quality of embryos. Several factors such as age of the women, concentration of sperm,

history of recurrent pregnancy loss, and prior failure of IVF known to effect the qualities of embryos. Objectively, quality of embryo can be evaluated by the use of pre implantation screening. This type of screening is used in order to detect genetic materials or morphological examinations [3]. To date, in Indonesia, qualities of embryo are checked based on morphologic appearance other than genetic screening. Morphological testing is still the chosen option to choose quality of embryos in short period of time. While preimplantation genetic screening is not being used routinely in Indonesia since it is expensive and couple needs to pay extra.

Previous studies shows pronucleus cells is one of the good predictor of evaluating zygote development. Around 5% from zygote came from In Vitro Fertilization method has more than two pronucleus (with highest rank is three pronucleus (3pn)). This abnormality is happened due to more than one penetration of sperm, or failure of extrusion from second polar body. The most common cause of this abnormalities is the penetration of more than two sperm into a single ovum, which causing embryos to have triploid chromosome of XXX, XXY, or XYY. This type of zygote will not undergo embryo transfer in IVF. The prevalence of 3PN is one to three percent of all embryos. Abnormalities are happening within the early stage of development, though some of the abnormalities might developed in later days [4].

Several examinations previously mention has purpose to evaluate anomalies within embryos before it get transferred inside a womb. However, there is no known risk factors that can cause conditions of 3pn zygote. Thus, knowing several factors in predicting the occurrence of three-pronuclear zygote can help much.

2. Method

The subjects of the study were 472 cycles in 449 subjects who underwent controlled ovarian hyperstimulation in IVF cycles at Yasmin Clinic of Dr. Cipto Mangunkusumo General Hospital, Jakarta, Indonesia between January 2013 and August 2014. The exclusion criteria were patients who did not follow the protocol of IVF to the completion and which the data is incomplete.

Data of medical records were recorded in the study forms which have been made specifically to facilitate data entry and analysis. The data were processed using IBM SPSS for windows version 20.

At this stage of analysis, we studied the relationship between two variables comprising the comparison between clinical variables, such as patient's age, type of infertility, causes of infertility in female and male, basal AMH level, and number of mature oocyte. Categorical variables were compared using Chi Square test and continuous variables were analyzed using Independent t-test, and statistically significant was considered when $p < 0.05$.

Characteristics		N (%)
Age	20-25	12 (2.5)
	26-30	76 (16.1)
	31-35	136 (28.8)
	36-40	164 (34.7)
	>40	84 (17.8)
Causes of Infertility	Female	437 (92.6)
	Male	35 (7.4)
AMH level	≤ 1.4	98 (20.8)
	>1.4	374 (79.2)
Number of Mature follicles	≤6	133 (28.2)
	>6	339 (71.8)
Female factor	Endometriosis	61 (13.9)
	PCOS	56 (12.9)
	Tubal factor	69 (15.7)
	DOR/Poor Responder	137 (31.1)
	Hyperprolactinemia	7 (1.6)
	Others	110 (25)

TABLE 1: Demographic data between IVF cycles (total of 472 cycles).

3. Results

There were four hundred seventy two cycles of IVF were performed in 449 couples undergoing IVF program from January 2013 and August 2014. The distribution of patients' ages during period of IVF program was between the ages of 20 until 48. Mean age of the patients was 35.5 years of age with mainly cause of infertility is female with poor responders.

Based on this study, it was found total of 38 cycles (8.05%) found to have 3pn embryos. Risk factors such as patient's age, number of mature oocyte and level or AMH were compared using Chi Square test (Table 2).

Based on Table 2, statistically significant findings to the incidence of 3pn is only level of AMH below 1,4. Figure 3 describes mean of each categorical dependent factor. Mean of age of patient whose having 3pn zygote is 36.49 compared to normal zygote of 35.40. Also number of mature follicle found to be more in 3pn zygotes with mean of 14.87. However, level of AMH in-patient present with 3pn zygote found to be lower compared to normal zygotes.

On Table 3, it is shown relative risk of level of AMH to the incidence of 3pn is 2.5 (CI 95% 1.56; 4.16).

Parameter	p-value
Age	0.08
Number of mature folicles	0.133
Level of AMH (< 1.4)	< 0.001

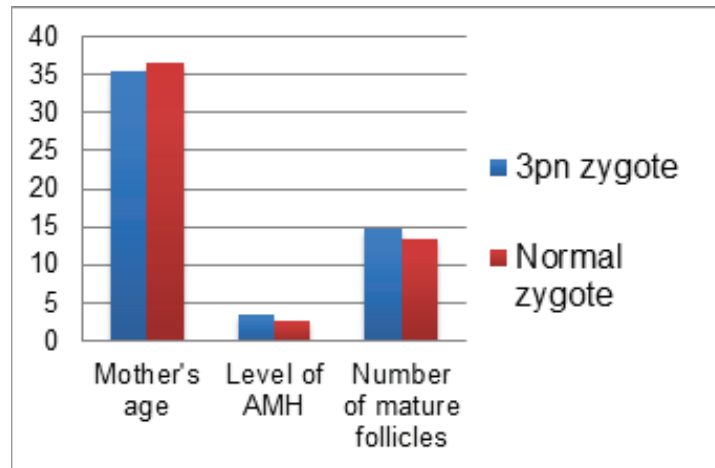TABLE 2: Value of p of risk factors influence the development of 3pn zygote.

Figure 1: Mean value of each risk factors in normal group and 3pn group

4. Discussion

Three pronuclear can happen when there is a fertilization of more than one sperm or there is failure meiosis of an oocyte. The cause of 3pn until today is still debatable. Some expert believe 3pn can be cause by maternal age or sperm abnormalities [5-7].

In this study, there were 472 cycles of IVF from 449 couple undergoing IVF cycles. There is 8.05 % of three pronuclear zygote can be found in this study. This number seems more than usual prevalence of 3pn worldwide. This study found there is no significant correlation between the incidence of 3pn with maternal age (p=0.08) and number of mature follicle (p = 0.133). This result is consistent with previous study by Li and friends in 2015, which found there is no significant correlation between maternal ages with the incidence of 3pn [5].

There is a statistically significant finding between levels of AMH with the incidence of 3pn zygotes (p < 0.01). In women, AMH is a hormone works in the cell trough two receptors that is AMH receptor type I and tipe II (AMHRI and AMHRII). AMH has two main function in female reproductive system, which is within primary recruitment,

AMH level	3PN Zygote	
	+	-
<1,4	22	76
>1,4	33	341

TABLE 3: Two by two table showing relationship of level of AMH with incidence of 3pn zygote.

AMH will inhibit primordial follicle recruitment into the growing pool and during cyclic recruitment, AMH will decrease the sensitivity of ovarian follicle to Follicle Stimulating Hormone (FSH), thus AMH known to be one of the factor which regulates the growing of the follicle, thus it will influence the oocyte qualities which then will influence the successfulness of IVF [8,9].

To date, there is no single study which trying to find the relationship between level of AMH with the incidence of 3pn zygote. However, level of AMH is proven to be use as an indicator to evaluate oocyte viability in IVF and low level of AMH can predict the occurrence of aneuploidy [10,11].

5. Conclusion

As a conclusion, level of AMH is known to be strong predictor of thee pronuclear zygote after IVF cycle. However, this preliminary study can be useful to conduct further studies in order to evaluate more risk factors of three-pronuclear zygote.

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