Research Article

The Number of LH Receptor could Predict the Success of Oocyte Maturity in the Process of In Vitro Maturation

Jumlah Reseptor LH dapat Memprediksi Keberhasilan Maturasi Oosit pada Proses Maturasi In Vitro

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Abstract

Objective: To evaluate the relationship between the number of LH receptor and the success of oocyte maturity in the process of in vitro maturation (IVM).

Method: This experimental study was conducted in the Permata Hati Infertility Clinical Laboratory, Dr. Sardjito General Hospital, Yogyakarta, with the samples of 300 oocytes obtained through collecting immature cow's oocytes from the abattoir and grouped the oocytes into 3 (three) groups based on the pattern of oocyte cumulus cells on the vesicle germinal stage 2 - 8 mm with three layers of cumulus cell. The sample of the cumulus cells from these three groups were taken and the LH receptor examination was done with immunohistochemistry. After that, the IVM process was performed to the three groups and its development for 24 hours was evaluated. Its maturation quality was evaluated with the emergence of the first polar body (1PB) and compared to the other groups and related to the number of LH receptor in the three groups.

Result: The result of this study indicated that the oocyte cumulus cells showed a difference of function during IVM process. The maturity rate in this study showed that the number of LH receptor was related to the morphological pattern of oocyte cumulus cells with oocyte maturity. The maturity of the cumulus cells which 100% covered the oocyte was higher than that of the cumulus cells which > 50% and < 30% covered the oocytes, namely, 74% compared to 60% and 12%. The result of this study also showed that the average number of LH receptors in the three groups (A, B, and C) was 183.4, 78.8, and 24.0 respectively. A significant difference was found in the three groups ($p < 0.0001$). When related to IVM maturity, this difference showed that the bigger number of oocyte cumulus cells influenced the oocyte maturity.

Conclusion: The number of LH receptor can be used as a prediction to determine the success of oocyte maturation in the process of in vitro maturation.

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Keywords: IVM, LH receptor, oocyte cumulus cell

Abstrak

***Tujuan**: Untuk menilai hubungan jumlah reseptor LH dengan keberhasilan maturitas oosit pada proses in vitro maturasi.*

***Metode**: Penelitian eksperimental dilakukan di Laboratorium Klinik Infertilitas Permata Hati RSUP. Dr. Sardjito Yogyakarta, dengan sampel 300 oosit. Dilaksanakan dengan cara mengumpulkan oosit sapi yang immatur yang didapat dari rumah potong hewan dan dikelompokkan menjadi 3 kelompok berdasarkan pola sel kumulus oosit pada stadium germinal vesicle 2-8 mm dengan tiga lapis sel kumulus. Pada ketiga kelompok ini diambil contoh sel kumulus dan dilakukan pemeriksaan reseptor LH dengan imunohistokimia. Selanjutnya ketiga kelompok dilakukan IVM dan dinilai perkembangannya selama 24 jam. Dinilai kualitas pematangannya dengan munculnya polar body pertama (1PB) dan dibandingkan dengan kelompok lain dan dihubungkan dengan jumlah reseptor LH pada ketiga kelompok.*

***Hasil**: Dari penelitian ini menunjukkan sel kumulus oosit menunjukkan perbedaan fungsi pada IVM yaitu angka maturitas dan pada penelitian ini menunjukkan jumlah reseptor LH berhubungan dengan pola morfologi sel kumulus oosit dengan maturitas oosit. Maturitas dari sel kumulus yang seluruhnya (100%) menutupi oosit lebih tinggi dibanding dengan sel kumulus yang sebahagian besar >50% menutupi oosit dan sel kumulus yang sebahagian kecil <30 menutupi oosit yaitu 74% dibanding 60% dan 12%. Pada penelitian ini menunjukkan bahwa jumlah rata-rata reseptor LH pada ketiga kelompok A, B dan C masing masing 183,4, 78,8, dan 24,0. Dijumpai perbedaan yang bermakna pada ketiga kelompak ($p<0,0001$). Bila hal ini kita hubungkan dengan maturitas IVM menunjukkan bahwa jumlah sel kumulus oosit yang lebih banyak mempengaruhi maturitas oosit.*

***Kesimpulan**: Jumlah reseptor LH dapat digunakan sebagai prediksi untuk menentukan keberhasilan maturasi oosit pada proses maturasi in vitro.*

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***Kata kunci**: IVM, reseptor LH, sel kumulus oosit*

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INTRODUCTION

Ovarium stimulation product in the current IVF program can be replaced by taking the immature oocytes and conducting in vitro maturaton (IVM). The main advantages of conducting IVM are to prevent the risk of ovarium hyperstimulation, to the ability minimize the cost and to reduce treatment complication.[1,2]

IVM in the immature oocytes has been conducted in the super ovulation cycle, natural cycle, and PCOS patients 2-5, 10-15% of the oocytes originally from the stimulated cycle are still immature and these oocytes can be processed through IVM to produce good mature oocytes.[4]

It was previously reported that the oocytes with various morphologic patterns of oocyte cumulus cell were obtained at the time of oocyte taking, either in the super ovulation cycle, natural cycle or PCO patients, namely, the oocyte at vesicle germinal stage with oocyte cumulus cell pattern.[6] This occured due to the angle of taking and the pressure of the vacuum during the ovum pick up. In fact, various morphologic pattern of oocyte cumulus cell in IVM process produced different ability to the oocyte maturity. Currently, there hasn't been many studies done on the oocyte cumulus cell morphology-related to oocyte maturity.

Oocyte maturity depends on the communication between follicular cell and the existence of FSH and LH receptors. Oocyte cumulus cells respond the FSH and LH and secrete various substances playing an important role in the nucleus and cytoplasm maturation. FSH is important for the development of in vivo preovulation follicle and to induce LH receptors.[4,7,8]

GVBD is initiated by the pre-ovulation surge of gonadotropin hormone (LH). Many potential factors have become the mediator controling the cumulus cell in GVBD. The great number of cyclic adenosine monophosphate (cAMP) and purin hypoxanthine in culture media prevents oocyte GVBD. Oocyte and cumulus cell are connected by gap junctions. The gap junction allows the regulator of molecules such as steroid, calcium ion, IP3 (inositol 1, 4,5 - triphosphate), cAMP, and purin to pass freely between oocyte cytoplasm and cumulus cell.[4]

The addition of LH (luteinizing hormone) into the culture media induces GVBD. The possibility of LH to induce GVBD is by an indirect action mediated by cumulus cells because LH receptor are not found in the oocyte.[4] The LH-involving mechanism induces the loss of communication between oocyte and cumulus cell that the flow of molecule regulator into the oocyte stops. The LH-induced GVBD may also be mediated by IP3/Ca2+.

This indicates that LH plays an important role in the further stage of follicle development, provides support for final maturation and dominant function of follicle, therefore, the existence of LH in the follicle before ovulation is an important contributor for the optimal follicle development which in the end produces healthy oocytes.[4]

METHOD

Various morphologic patterns of cumulus cell and oocyte are estimated to be providing a different ability to oocyte maturity.

After that, LH receptor in the oocyte cumulus cell is a determining factor for oocyte formation with optimal maturity. In accordance with the theoretical basis, the research conceptual framework can be described in the following scheme:

This experimental study was conducted in the Permata Hati Infertility Clinical Laboratory, Dr. Sardjito General Hospital, Yogyakarta, with the samples of 300 oocytes derived from cows.

The cow's immature oocytes used in this study were obtained from the abattoir and then grouped into 3 (three) groups based on the pattern of oocyte cumulus cell at the vesicle germinal stage of 2 - 8 mm with three layers of cumulus cell. Group A consisted of the cumulus cell which 100% covered the oocyte, Group B consisted of the cumulus cell which > 50% covered the oocyte, and Group C consisted of the cumulus cell which < 30% covered the oocyte. IVM was conducted to the three groups by using TCM plus HMG 0.1 IU/ml plus Follicle Fluid 10%.

To check the number of LH receptors in oocyte cumulus cell, 5 sample oocytes were taken from the respective three patterns of oocyte cumulus cell and the denuded through repeated mechanical suctions by using pipette. Then, the LH receptor was examined by using immunohistochemistry (Monoclonal Antibody, Termo Scientific).

The quality of oocyte maturity was valued 24 hours after the emergence of IPB. The maturity quality of the oocytes that had reached the MII maturity was evaluated and compared to the other groups and then related to the expression of LH receptors in the three groups. To obtain the valid data, data collection was done through documentation observation. Observation in this study was a direct observation on the IVM procedures. Documentation covered the data collection supporting the result of observation including the develop-

ment of ovum of each group and image documentation.

The data collected were then processed and analyzed. The level of significance used p value = 0.05 (95%). Through univariate analysis, the data obtained were descriptively analyzed to show the characteristics of research population. Bivariate analysis was done to evaluate the relationship between 2 (two) variables, namely, independent and dependent variables. The analysis was done through strata 6.

RESULT

This study was conducted based on the samples of 300 oocytes which were grouped into 3 (three) groups based on the pattern of oocyte cumulus cell at the vesicle germinal stage of 2 - 8 mm with three layers of cumulus cell. Group A consisted of the cumulus cell which 100% covered the oocyte, Group B consisted of the cumulus cell which > 50% covered the oocyte, and Group C consisted of the cumulus cell which < 30% covered the oocyte.

Table 1. Comparison of Oocyte Maturity (MII) of the Three Groups

Parameter	Group A	Group B	Group C	Total
MII	74	60	12	146
Not matur	26	40	88	154
Total	100	100	100	300

Table 2. The Difference of LH Receptor of Oocyte Cumulus Cell of the Three Groups

Pattern of cumulus cells	n	X ± SD	95 % CI for men	p
Group A	5	183.40 ± 38.132	(134.81;231.91)	
Group B	5	78.80 ± 17.138	(57.52;100.08)	0.0001*
Group C	5	24.00 ± 13.812	(6.7;41.25)	

anova one way
** significant*

The result of ANOVA test showing p < 0.05 means that there are different average numbers of LH receptors in the pattern of cumulus cell. The different average can be seen from the result of LSD multiple comparative tests as follows:

Group A and Group B : p = 0.0001

Group A and Group C : p = 0.0001

Group B and Group C : p = 0.0001

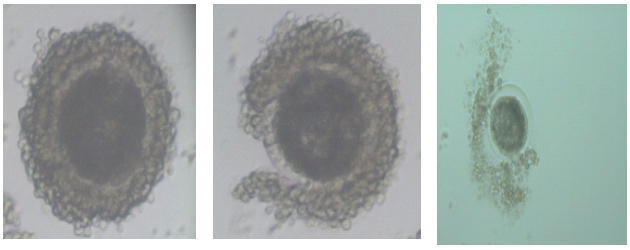

Figure 1. Description of the pattern of cumulus cell covering the oocyte of A, B, and C group

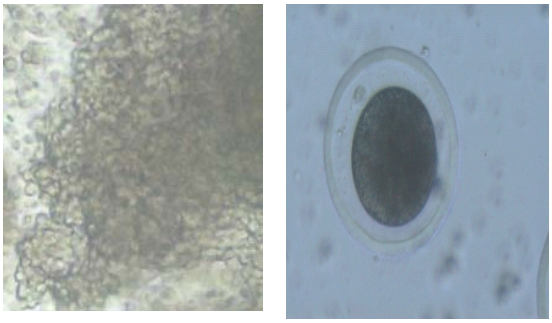

Figure 2. Cumulus cell and Oocyte MII

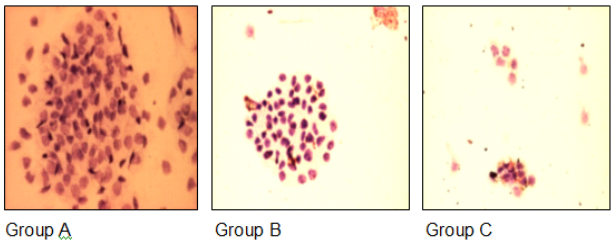

Figure 3. Number of LH receptor of oocyte cumulus cell of the three colorations using immunohistochemistry

DISCUSSION

The result of this study showed that the oocyte cumulus cell indicated a different function in IVM in the form of maturity rate and the number of LH receptor is related to the morphologic pattern of oocyte cumulus cell with oocyte maturity.

The maturity of cumulus cell morphology covering the whole oocytes (100%) is higher compared to that of cumulus cell morphology covering most of the oocytes (> 50%) and small part of the oocytes (< 30%) with the respective ratio of 74%, 60%, and 12%.

This result is in line with that of the study conducted by Cha and Chian (1998),[4] reporting that the difference of the interval of GVBD on oocytes GV stage, the IVF patients with super ovulation, the unstimulated IVF patients, produced by the differ-

Table 3. The Prediction of the Relationship of the Number of LH Receptor with Maturity Presentation

Group	Number of receptor LH	CI 95 % Average number of Average		Presentation of Oocyte maturation	CI 95% the Percentage of oocyte maturation	
	X ± SD	Upper	Lower		Upper	Lower
A	183.40 ± 38.132	134.81	231.91	74 %	65.3 %	82.7%
B	78.80 ± 17.138	57.52	100.08	60 %	50.3 %	69.7%
C	24.00 ± 13.812	6.7	41.25	12 %	5.6 %	18.4 %

ent patterns of oocyte cumulus cell on oocyte GV stage.

The granulose cell connected by the vast gap junction tissues effectively links them into a functional and integrated system. This particular cell junction is important for metabolic exchange and small molecular transport between these adjacent cells. In addition, granulose cell extends its cytoplasmic process through pellucida zone to form gap junction with oocyte plasma membrance. Cyclic Adenosin Monophosphate (cAMP) produced by granulose cell, can be one of the important factors passing into the oocyte through gap junction to maintain the oocyte to be outside the maturation stopping stage.[4,8]

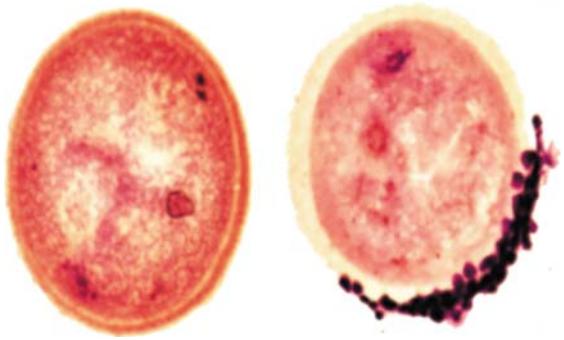

Figure 4. There is no LH receptor in the oocyte

Gap junction consists of hexameric protein composition called connexin. Connexin-37 and Connexin-43 are the two important follicle connexin. Connexin-37 is reported as the main connexin of oocyte, while Connexin-43 as the main connexin of granulose cell. Consequently, the communication between granulose cell and oocyte occurs through heterologous gap junction, in which the intergranulose cell communication through gap junction is by means of homologous complexes. FSH induces the expression of Connexin-43 in granulose cell while the ovulatory LH surge supresses the mRNA of Connexin-43 and causes the emergence of post-translational modification resulting in the loss of Connexin-43 protein and then the separation of inter-granulose cell gap junction tissues and between granulose cell and oocyte.[9,10]

The primary steroid hormone produced by preovulatory granulose cell is estradiol. This hormone synthesis needs mutual cooperation with theca cell producing immediate precusor for reaction of aromatization. The control of this process is under the LH arrangement reacting to the elements of theca and the FSH arrangement reacting to the granulose compartment. The 2 cell-2 gonadotropin Model is the appropriate example of the integrated function of the cellular component of different follicles.[4,9,10]

GVBD is an indirect action mediated by cumulus cell due to the absence of LH receptor in the oocyte (Dekel, 1988).[4] LH-involved mechanism induces the loss of communication between oocyte and cumulus cell that the flow of molecule regulator into the oocyte stops. LH-induced GVBD may also be mediated by IP3/Ca2+ path.

This showed that LH plays an important role in the further stage of follicle development, provides support for the end maturation and dominant function of follicle, therefore, the existence of LH in the follicle before the ovulation is an important contributor for an optimal follicle development which eventually produces the healthy oocytes.

This study showed that the average number of LH receptors in the three groups (A, B, C) is 183.4, 78.8, and 24.0 respectively. A significant difference is found in the three groups ($p < 0.0001$).

In relation to IVM maturity, the result above showed that the bigger number of oocyte cumulus cell has influenced the oocyte maturity. In group A, the maturity of the cumulus cell which 100% covered oocyte was 74%. In group B, the maturity of the cumulus cell which > 50% covered oocyte was

60%. In group C, the maturity of the cumulus cell which < 50% covered oocyte was 12%.

The result of this study is in line with that of the study conducted by Cha et al in 1994 on the immature human oocytes who successively used follicle fluid and peritoneal fluid as the supplement in the media of maturation with the maturation rate of 63% and 60%.

In this study, it can also be proven with immunohistochemistry that oocyte does not have any LH receptor.

The conclusion is that LH receptor can be used as the prediction to determine the success of oocyte maturity in the process of in vitro maturation.

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