3. Menguji Doktor

KODE K05

DESKRIPSI	:	Penguji Tertutup	
		a.n Ninik Darsini, dr., M.Biomed	Halaman
BUKTI	:	SK Dekan No 223/UN3.1.1/HK.04/2020, tanggal 26 Juni 2020 …….	02
		Bukti kinerja yaitu hal sampul, hal pengesahan dll …..	05

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SALINAN

KEPUTUSAN
DEKAN FAKULTAS KEDOKTERAN
NOMOR 223/UN3.1.1/HK.04/2020

TENTANG

PANITIA UJIAN TAHAP PERTAMA (TERTUTUP)
PROGRAM DOKTOR PROGRAM STUDI ILMU KEDOKTERAN
FAKULTAS KEDOKTERAN ATAS NAMA NINIK DARSINI, dr., M.Biomed.

DEKAN FAKULTAS KEDOKTERAN,

Menimbang :

a. bahwa sehubungan dengan telah siap dilakukan ujian tahap pertama (tertutup) Program Doktor Program Studi Ilmu Kedokteran Fakultas Kedokteran, maka perlu dibentuk panitia ujian tahap pertama (tertutup) tersebut;

b. bahwa nama-nama yang tersebut di bawah ini telah memenuhi syarat dan bersedia untuk diangkat sebagai panitia ujian dimaksud;

c. bahwa berdasarkan pertimbangan sebagaimana dimaksud pada huruf a dan huruf b, perlu menetapkan Keputusan Dekan Fakultas Kedokteran tentang panitia ujian tahap pertama (tertutup) Program Doktor Program Studi Ilmu Kedokteran Fakultas Kedokteran.

Mengingat :

1. Undang-Undang Nomor 20 Tahun 2003 tentang Sistem Pendidikan Nasional (Lembaran Negara Republik Indonesia Tahun 2003 Nomor 78, Tambahan Lembaran Negara Nomor 4301);
2. Undang-Undang Republik Indonesia Nomor 14 Tahun 2005 tentang Guru dan Dosen (Lembaran Negara Republik Indonesia Nomor 157, Tambahan Lembaran Negara Nomor 4586;
3. Undang-Undang Nomor 12 Tahun 2012 tentang Pendidikan Tinggi (Lembaran Negara Republik Indonesia Tahun 2012 Nomor 158, Tambahan Lembaran Negara Nomor 5336);
4. Undang-Undang Nomor 5 Tahun 2014 tentang Aparatur Sipil Negara (Lembaran Negara Republik Indonesia Tahun 2014 Nomor 06, Tambahan Lembaran Negara Nomor 5494);
5. Peraturan Pemerintah Republik Indonesia Nomor 57 Tahun 1954 tentang Pendirian Universitas Airlangga Di Surabaya sebagaimana telah diubah dengan Peraturan Pemerintah Nomor 3 Tahun 1955 tentang Pengubahan Peraturan Pemerintah Nomor 57 Tahun 1954. (Lembaran Negara Republik Indonesia Tahun 1954 Nomor 99 Tambahan Lembaran Negara Nomor 695 juncto Lembaran Negara Republik Indonesia Tahun 1955 Nomor 4 Tambahan Lembaran Negara Nomor 748);

6. ...

6. Peraturan Pemerintah Nomor 4 Tahun 2014 tentang Penyelenggaraan Pendidikan Tinggi dan Pengelolaan Perguruan Tinggi. (Lembaran Negara Republik Indonesia Tahun 2014 Nomor 16, Tambahan L embaran Negara Nomor 5500);
7. Peraturan Pemerintah Nomor 30 Tahun 2014 tentang Statuta Universitas Airlangga. (Lembaran Negara Republik Indonesia Tahun 2014 Nomor 100, Tambahan Lembaran Negara Nomor 5535);
8. Peraturan Rektor Universitas Airlangga Nomor 27 Tahun 2018 tentang Peraturan Pendidikan Universitas Airlangga;
9. Peraturan Rektor Universitas Airlangga Nomor 21 Tahun 2014 tentang Pedoman Pendidikan Program Doktor (S3) Universitas Airlangga;
10. Keputusan Rektor Universitas Airlangga Nomor 1947/H3/KR/2011 tentang Penetapan Ruang Lingkup Program Studi dalam Kategori Monodisiplin, Interdisiplin dan Multidisiplin untuk Pengelolaan Program Magister dan Program Doktor;
11. Keputusan Rektor Universitas Airlangga Nomor 1732/UN3/2015 tentang Pengangkatan Dekan Fakultas dan Direktur Sekolah Pascasarjana Periode 2015-2020.

MEMUTUSKAN:

Menetapkan : KEPUTUSAN DEKAN FAKULTAS KEDOKTERAN TENTANG PANITIA UJIAN TAHAP PERTAMA (TERTUTUP) PROGRAM DOKTOR PROGRAM STUDI ILMU KEDOKTERAN FAKULTAS KEDOKTERAN ATAS NAMA NINIK DARSINI, dr., M.Biomed.

PERTAMA : Membentuk panitia ujian tahap pertama (tertutup) Program Doktor Program Studi Ilmu Kedokteran Fakultas Kedokteran atas nama Ninik Darsini, dr., M.Biomed yang dilaksanakan pada tanggal, 26 Juni 2020 dengan susunan nama-nama sebagai berikut:

Ketua : Prof. Dr. Hendy Hendarto, dr., Sp.OG(K)
Anggota :
1. Prof. Win Darmanto, Drs., M.Si., Ph.D
2. Aucky Hinting, dr., Ph.D., Sp.And
3. Prof. Dr. Ir. Trinil Susilawati, MS
4. Dr. Rina Yudiwati, dr., MS
5. Dr. H. Budi Utomo, dr., M.Kes
6. Dr. Gondo Mastutik, dr., M.Kes

KEDUA : Dalam menjalankan tugasnya sebagaimana dimaksud dalam diktum PERTAMA, berpedoman pada peraturan dan ketentuan yang berlaku serta mempertanggungjawabkan tugasnya kepada Dekan Fakultas Kedokteran.

KETIGA : Biaya untuk keperluan tersebut dibebankan pada dana Rencana Kegiatan dan Anggaran Tahunan (RKAT) Fakultas Kedokteran.

KEEMPAT: ...

KEEMPAT : Keputusan ini mulai berlaku pada tanggal ditetapkan.

Ditetapkan di Surabaya
pada tanggal 26 Juni 2020

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DISERTASI

POTENSI EKSPRESI *MICRO-RNA (miRNA) hsa-mir-34 FAMILY (hsa-mir-34b-3p, hsa-mir-34b-5p, hsa-mir-34c-3p, hsa-mir-34c-5p)* PADA SPERMATOZOA PRIA OLIGOZOOSPERMIA SEBAGAI KANDIDAT BIOMARKER DIAGNOSIS INFERTILITAS IDIOPATIK

NINIK DARSINI

PROGRAM STUDI ILMU KEDOKTERAN JENJANG DOKTOR
FAKULTAS KEDOKTERAN UNIVERSITAS AIRLANGGA
SURABAYA
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DISERTASI

POTENSI EKSPRESI *MICRO-RNA (miRNA) hsa-mir-34 FAMILY (hsa-mir-34b-3p, hsa-mir-34b-5p, hsa-mir-34c-3p, hsa-mir-34c-5p)* PADA SPERMATOZOA PRIA OLIGOZOOSPERMIA SEBAGAI KANDIDAT BIOMARKER DIAGNOSIS INFERTILITAS IDIOPATIK

DISERTASI
Untuk memperoleh Gelar Doktor
Dalam Program Studi Ilmu Kedokteran Jenjang Doktor
pada Fakultas Kedokteran Universitas Airlangga dan
dipertahankan di hadapan Panitia Ujian Akhir Tahap 1 (Tertutup)

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PROGRAM STUDI ILMU KEDOKTERAN JENJANG DOKTOR
FAKULTAS KEDOKTERAN UNIVERSITAS AIRLANGGA
SURABAYA
2020

LEMBAR PENGESAHAN

DISERTASI

POTENSI EKSPRESI *MICRO-RNA (miRNA) hsa-mir-34 FAMILY*
(hsa-mir-34b-3p, hsa-mir-34b-5p, hsa-mir-34c-3p, hsa-mir-34c-5p)
PADA SPERMATOZOA OLIGOZOOSPERMIA SEBAGAI KANDIDAT
BIOMARKER DIAGNOSIS INFERTILITAS PRIA IDIOPATIK

TELAH DISETUJUI
PADA TANGGAL 12 JUNI 2020

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oleh panitia penguji Ujian Akhir Tahap 1 (Tertutup)
pada tanggal 26 Juni 2020

Panitia Penguji

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SUMMARY

THE POTENTIAL OF MICRO-RNA EXPRESSION hsa-mir-34 FAMILY (hsa-mir-34b-3p, hsa-mir-34b-5p, hsa-mir-34c-3p, hsa-mir-34c-5p) IN OLIGOZOOSPERMIA AS BIOMARKERS CANDIDATES FOR DIAGNOSIS OF IDIOPATHIC INFERTILITY

About 60-75% of infertility cases are idiopathic, suspected genetic or epigenetic factors can be one of the causes. Changes in microRNA expression (miRNA) found in oligozoospermia are thought to be one of the causes of male infertility. The role of miRNA is associated with the process of spermatogenesis. Abnormalities in miRNA, both overexpression and deletion, can affect various cellular processes, including spermatogenesis. Changes in miRNA expression contribute to the failure of the spermatogenesis process (spermatogenic failure). The poor quality of spermatozoa is thought to be caused by differences in miRNA expression in the process of spermatogenesis.

MicroRNA (miRNA) is a small molecule of the noncoding RNA family, single stranded and composed of 22-24 nucleotides. The term miRNA was only used in 2001. miRNA was first discovered in 1993 in the research of Lee et al when doing research on lin-4 gene sequences on nematodes. miRNA plays a role in regulating the stability and translation of mRNA. Until now more than 1500 miRNAs of the human genome have been found and are located on all chromosomes, miRNA can be found not only in cells, but also in saliva, blood, vaginal fluid and semen ejaculate. MiRNA mapping has not yet been thoroughly researched by scientists Various studies have tried to find miRNA candidates who play a significant role in the process of spermatogenesis in the hope that they can be used as non-invasive biomarker candidates for the diagnosis of idiopathic male infertility

In oligozoospermia, the hsa-mir-34 family is one candidate for miRNA. They are hsa-mir-34b-3p, hsa-mir-34b-5p, hsa-mir-34c-3p and hsa-mir-34c- 5p. The expression of hsa-mir-34 family is estimated to be a strong candidate as a determinant of the quality of the spermatozoa produced. In the mechanism of the process of spermatogenesis, hsa-mir-34 family is thought to be an inhibitor in the maturation process, so the number of spermatozoa produced is below normal. Until now in Indonesia there has been no research on the expression of hsa-mir-34 family in oligozoospermia.

This research is an analytic study with case-control design. The case group was an oligozoospermia male with a concentration of spermatozoa below 15 million/ml, while the control group was a normozoospermia with a concentration of spermatozoa upper or equal to 15 million/ml. The location of research was conducted at the Andrology, Embryology and Molecular Genetics Laboratory of the Department of Medical Biology, Faculty of Medicine, Airlangga University, RSKI Laboratory and ITD (Institute Tropical Disease) Airlangga University, Surabaya. The number of research samples obtained 66 volunteers, with details of 31 volunteers oligozoospermia and 35 volunteers normozoospermia.

The research included semen analysis, spermatozoa isolation, miRNA isolation, and RT-qPCR examination. Isolation of spermatozoa using the Somatic Cell Lysis Buffer (SCLB) method of Goodrich. Isolation and purification of miRNA using the Hybrid-R ™ miRNA catalog kit no. 325-150 from GeneAll Biotechnology, Seoul, Korea. Measurement of miRNA levels using the ND-1000 nanodrop spectrophotometer. RT-qPCR examination uses one step method with Qiagen Rotor-Gene Q machine. The master mix reagent uses the NEXproTM qRT PCR 2X master mix (EVA) master mix, catalog no. NexQ-7200, Korea. Primary hsa-mir-34b-3p and hsa-mir-34b-5p are primary sets from Cohesion Biosciences, catalog CPK1057, UK, while Primers U6, hsa-mir-34c-3p and hsa-mir-34c-5p are the primary design sequences from Bioneer AccuOligo®, catalog S-1001, Bioneer Corporation, Korea.

The results showed that voluntary age was normally distributed and there were no differences in voluntary age between oligozoospermia and normozoospermia. Ejaculate fluid volume is normally distributed and there is no difference between oligozoospermia and normozoospermial. The test results showed that voluntary age and ejaculate fluid volume before spermatozoa isolation were carried out under homogeneous conditions. PH and viability values also indicate homogeneous conditions, although the distribution is not normal, there is no difference between oligozoospermia and normozoospermia. Concentration, total sperm cell (TSC) before isolation, total sperm cell (TSC) after isolation and recovery rate found a significant difference between oligozoospermia and normozoospermia. Characteristics of miRNA isolates obtained miRNA levels with varying values, while the level of purity of miRNA obtained optimal results in the range of 1.8-2.2.

RT-qPCR validation results obtained optimal Qiagen Rotor-Gene Q machine settings with hot start settings at a temperature of 48°C 20 minutes, followed by a temperature of 95°C 10 minutes, denaturation temperature of 95°C 20 seconds, annealing temperature varies according to the results of validation 40 seconds and extensions 72°C 60 seconds. The primary annealing temperature of U6 and hsa-mir-34c-5p is 59°C, the annealing temperature of hsa-mir-34b-3p and hsa-mir-34b-5p are 60°C, while hsa-mir-34c-5p is obtained annealing temperature 58°C .

The results of running RT-qPCR on the hsa-mir-34 family showed that the relative expression level of hsa-mir-34b-3p in oligozoospermia was lower (down regulation) of 0.91 and the relative expression of hsa-mir-34b-5p was also obtained more low (down regulation) of 0.64 compared to normozoospermia. The relative expression of hsa-mir-34c-3p in oligozoospermia was found to be higher (up regulation) by 1.39 and the relative expression of hsa-mir-34c-5p was also higher (up regulation) 2.06 compared to normozoospermia.

Based on the literature, reduced expression of hsa-mir-34b-3p and hsa-mir-34b-5p, and increased expression of hsa-mir-34c-3p and hsa-mir-34c-5p inhibits the formation of p53 protein, so that cell proliferation is inhibited and accelerated apoptosis. These conditions have an impact on spermatogenesis and cause a decrease in the concentration of spermatozoa produced (oligozoospermia). Another mechanism is the PLCX3 gene that encodes the PLCX3 protein. Increased expression of hsa-mir-34c-3p inhibits the formation of the PLCX3 protein, thereby inhibiting spermatogenesis and causing decreased production of spermatozoa (oligozoospermia).

This study concludes that the expression of hsa-mir-34 family (hsa-mir-34b-3p, hsa-mir-34b-5p, hsa-mir-34c-3p and hsa-mir-34c-5p) in oligozoospermia has a potential as biomarker candidate for idiopathic infertility diagnosis in Indonesia with down regulation mechanism on hsa-mir-34b-3p and hsa-mir-34b-5p, and up regulation on hsa-mir-34c-3p and hsa-mir-34c-5p.

ABSTRAK

POTENSI EKSPRESI *MICRO-RNA (miRNA) hsa-mir-34 FAMILY (hsa-mir-34b-3p, hsa-mir-34b-5p, hsa-mir-34c-3p, hsa-mir-34c-5p)* PADA SPERMATOZOA PRIA OLIGOZOOSPERMIA SEBAGAI KANDIDAT BIOMARKER DIAGNOSIS INFERTILITAS IDIOPATIK

Tujuan : Penelitian ini secara umum bertujuan menganalisis potensi ekspresi *hsa-mir-34 family (hsa-mir-34b-3p, hsa-mir-34b-5p, hsa-mir-34c-3p* dan *hsa-mir-34c-5p)* pada spermatozoa pria oligozoospermia sebagai kandidat biomarker diagnosis infertilitas idiopatik di Indonesia.

Material dan metode : Penelitian ini merupakan penelitian analitik dengan jenis penelitian *case-control*, kelompok kasus adalah sampel dari spermatozoa pria oligozoospermia dan kelompok kontrol dari spermatozoa pria normozoospermia. Jumlah sampel penelitian didapatkan 66 volunter dari beberapa klinik fertilitas di Surabaya dengan perincian 31 volunter oligozoospermia dan 35 volunter normozoospermia. Tahap penelitian meliputi tahap analisis semen, tahap isolasi spermatozoa, isolasi *miRNA*, dan pemeriksaan RT-qPCR.

Hasil : Karakteristik analisis semen tidak didapatkan perbedaan signifikan pada parameter umur, volume, pH dan viabilitas antara oligozoospermia dan normozoospermia. Konsentrasi awal. TSC awal, TSC akhir sesudah isolasi dan tuaian (*recovery rate*) didapatkan perbedaan signifikan antara oligozoospermia dan normozoospermia. Karakteristik isolat *miRNA* didapatkan kadar *miRNA* dengan tingkat kemurnian yang optimal dalam kisaran 1,8-2,2. Hasil validasi RT-qPCR suhu *annealing* U6 dan *hsa-mir-34c-5p* didapatkan suhu 59°C, suhu *annealing hsa-mir-34b-3p* dan *hsa-mir-34b-5p* didapatkan suhu 60°C, sedangkan *hsa-mir-34c-3p* didapatkan suhu *annealing* 58°C. Hasil RT-qPCR *hsa-mir-34 family* didapatkan ekspresi *hsa-mir-34b-3p* pada spermatozoa pria oligozoospermia lebih rendah (*down regulation*) sebesar 0,91 dan ekspresi *hsa-mir-34b-5p* juga didapatkan lebih rendah (*down regulation)* sebesar 0,64. Ekspresi *hsa-mir-34c-3p* pada spermatozoa pria oligozoospermia didapatkan lebih tinggi (*up regulation*) sebesar 1,39 dan ekspresi *hsa-mir-34c-5p* juga didapatkan lebih tinggi (*up regulation*) 2,06 dari normozoospermia.

Simpulan : ekspresi *hsa-mir-34 family (hsa-mir-34b-3p, hsa-mir-34b-5p, hsa-mir-34c-3p* dan *hsa-mir-34c-5p)* pada spermatozoa pria oligozoospermia dapat digunakan sebagai kandidat biomarker diagnosis infertilitas idiopatik di Indonesia dengan mekanisme *down regulation* pada *hsa-mir-34b-3p* dan *hsa-mir-34b-5p*, serta *up regulation* pada *hsa-mir-34c-3p* dan *hsa-mir-34c-5p*.

Kata kunci : *miRNA*, *hsa-mir-34 family*, oligozoospermia, infertilitas idiopatik

ABSTRACT

THE POTENTIAL OF EXPRESSION hsa-mir-34 FAMILY (hsa-mir-34b-3p, hsa-mir-34b-5p, hsa-mir-34c-3p, hsa-mir-34c-5p) IN OLIGOZOOSPERMIA AS BIOMARKERS CANDIDATES FOR THE DIAGNOSIS OF IDIOPATHIC INFERTILITY

Objective : This study generally aims to analyze the potential of expression hsa-mir-34 family (hsa-mir-34b-3p, hsa-mir-34b-5p, hsa-mir-34c-3p and hsa-mir-34c-5p) in oligozoospermia as biomarkers candidates for the diagnosis of idiopathic infertility in Indonesia.

Material and methods : The study design was an analytic study with a case-control design. The case groups were samples of oligozoospermia men and control groups of normozoospermia men. The samples were obtained by 66 volunteers from several fertility clinics in Surabaya, 31 oligozoospermia and 35 normozoospermia. This research phase includes semen analysis, spermatozoa isolation, miRNA isolation and RT-qPCR examinations.

Results : The characteristics of semen analysis did not reveal significant differences in age, volume, pH and viability parameters between oligozoospermia and normozoospermia. Concentration and TSC before isolation, TSC after isolation and recovery rate were significant differences between oligozoospermia and normozoospermia. Characteristics of miRNA isolates obtained levels of miRNA with optimal purity in the range of 1.8-2.2. The validation results of RT-qPCR annealing temperature U6 and hsa-mir-34c-5p obtained temperature 59°C, annealing temperature hsa-mir-34b-3p and hsa-mir-34b-5p obtained temperature 60°C, and hsa-mir-34c- 3p obtained annealing temperature 58°C. Results of RT-qPCR hsa-mir-34 family obtained hsa-mir-34b-3p expression in oligozoospermia men were lower (down regulation) by 0.91 and hsa-mir-34b-5p expression was also found to be lower (down regulation) amounted to 0.64. The expression of hsa-mir-34c-3p in oligozoospermia male spermatozoa was higher (up regulation) by 1.39 and the expression of hsa-mir-34c-5p was also higher (up regulation) 2.06 than normozoospermia.

Conclusion : Expression of hsa-mir-34 family (hsa-mir-34b-3p, hsa-mir-34b-5p, hsa-mir-34c-3p and hsa-mir-34c-5p) in oligozoospermia men can be used as a biomarker candidate for diagnosis idiopathic infertility in Indonesia with the mechanism of down regulation in hsa-mir-34b-3p and hsa-mir-34b-5p, and up regulation in hsa-mir-34c-3p and hsa-mir-34c-5p.

Keywords : miRNA, hsa-mir-34 family, oligozoospermia, idiopathic infertility