

# Pengaruh Suplementasi Spermin Dalam Diet Selama Masa Gestasi Terhadap Maturasi Ileum Janin *Oryctolagus Cuniculus*: Kajian Pada Protein Penyusun Tight Junction Dan Struktur Vili Usus = Effect of dietary spermine supplementation during gestation period on ileal maturation in premature *Oryctolagus cuniculus* fetus : An investigation on proteins composing tight junction and intestinal villi structure

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## Abstrak

**Latar Belakang.** Kelahiran bayi prematur di Indonesia menempati peringkat ke-5 di dunia. Sebanyak 50% bayi prematur memiliki risiko kematian yang lebih tinggi akibat infeksi, dimana 90% diantaranya disebabkan oleh infeksi saluran cerna. Hal ini dikaitkan dengan imaturitas saluran cerna. Spermin, senyawa poliamin, diketahui berperan penting dalam proliferasi, pertumbuhan, serta diferensiasi sel. Pada saluran cerna, spermin diketahui berinteraksi dengan protein penyusun barier usus dan berperan penting dalam penyembuhan luka serta sistem imun. Belum pernah dilakukan penelitian mengenai efek spermin selama masa gestasi, sehingga efek spermin terhadap maturasi usus in utero menjadi penting untuk diketahui. **Tujuan.** Untuk mengetahui pengaruh suplementasi spermin dalam diet terhadap maturasi protein tight junction selama masa gestasi yang berbeda pada kelinci.

**Metode Penelitian.** Desain penelitian merupakan studi analitik eksperimental menggunakan hewan coba kelinci New Zealand White (*Oryctolagus cuniculus*), yang dilakukan di Laboratorium Hewan Coba Puslitbang Biomedis dan Teknologi Dasar Kesehatan Badan Litbangkes Kementerian Kesehatan Republik Indonesia, Departemen Histologi FKUI, Departemen Biokimia dan Biologi Molekuler FKUI, dan Laboratorium Terpadu FKUI mulai dari bulan Oktober 2018 - September 2019. Setelah dilakukan anestesi umum, sampel jaringan usus halus janin kelinci diambil dan dibagi dalam 6 kelompok yang terdiri dari kelompok perlakuan (dengan suplementasi spermin 20 mg/kgBB) dan kelompok tanpa perlakuan (tanpa suplementasi spermin), masing-masing kelompok berasal dari induk kelinci dengan usia gestasi 24 hari, 26 hari, dan 28 hari. Jumlah masing-masing kelompok adalah 4 induk gestasi dengan berat badan berkisar antara 3-3,5 kg dengan janin berkisar 5-9 ekor per induk gestasi. Jaringan usus halus dari setiap kelompok diambil untuk pemeriksaan biokimia menggunakan teknik ELISA untuk  $\beta$ -actin,  $\beta$ -catenin, dan occludin, serta pemeriksaan histomorfologi dengan pewarnaan hematoxyllin-eosin. Analisis statistik menggunakan uji Mann-Whitney U, uji Chi Square dengan uji Fisher untuk data proporsi, dan uji korelasi Spearman untuk data numerik.

**Hasil.** Tidak ditemukan perbedaan konsentrasi  $\beta$ -actin,  $\beta$ -catenin, dan occludin antar kelompok perlakuan dan non perlakuan. Pada kelompok perlakuan dan tidak pada kelompok non-perlakuan, ditemukan adanya korelasi positif bermakna antara konsentrasi  $\beta$ -actin dan  $\beta$ -catenin,  $\beta$ -actin dan occludin, serta  $\beta$ -catenin dan occludin. Hasil skoring maturasi barier pada kelompok dengan suplementasi spermin pada usia gestasi 24 dan 26 hari mendekati kelinci aterm.

**Simpulan.** Suplementasi spermin dalam diet selama masa gestasi memperbaiki interaksi antar molekul tight junction pada janin kelinci prematur.

.....Background. Indonesia is ranked 5th as a country with premature births. Half of the premature infants

carry higher risks of death, in which 90% are due to gastrointestinal tract infection — these cases associated with the immaturity of the gastrointestinal tract system. Spermine is a polyamine molecule known for its essential role in cell proliferation, growth, and differentiation. Previous studies reported that spermine could interact with junctional proteins in the small intestine and responsible for maintaining the intestinal barrier integrity. However, to date, the efficacy of dietary spermine supplementation during the gestation period in utero remains unclear. Thus, an investigation is required. The purpose of the present study is to investigate the mechanism of spermine in improving intestinal villi barrier in premature rabbit fetus.

**Aim.** To investigate the effect of spermine supplementation in diet on the maturation of intestinal tight junction proteins during different rabbit gestation period.

**Method.** This study was an analytical, experimental study on New Zealand White Rabbits (*Oryctolagus cuniculus*) as animal models, performed at Laboratorium Hewan Coba Puslitbang Biomedis dan Teknologi Dasar Kesehatan Badan Litbangkes Kementerian Kesehatan Republik Indonesia, Departments of Histology FKUI, Department of Biochemistry and Molecular Biology FKUI, and Integrated Laboratory FKUI, from October 2018 until September 2019. Following general anesthesia, rabbit fetal intestinal specimens were taken and divided into six groups, consisting of groups given the intervention (spermine 20 mg/kg BW supplementation) and groups without intervention, each group based on the gestation period of 24 days, 26 days, and 28 days.  $\beta$ -actin,  $\beta$ -catenin, and occludin of ileal portion were determined and was stained by hematoxyllin-eosin for histomorphological assessment. Statistical analysis was carried out using the Mann-Whitney U test, Chi-Square test with Fisher test for data proportion, and Spearman's rank correlation for numeric data.

**Results.** There was no significant difference for  $\beta$ -actin,  $\beta$ -catenin, dan occludin concentration between groups with- and without spermine supplementation. Significantly positive correlation was obtained in the groups with- but not in the groups without spermine supplementation, between concentration of  $\beta$ -actin and  $\beta$ -catenin,  $\beta$ -actin and occludin, as well as  $\beta$ -catenin and occludin. The barrier scoring of ileal histomorphology in groups with spermine supplementation at gestation period of 24 dan 26 days were similar to a mature fetus.

**Conclusion.** Spermine supplemented diet given during the gestation period improves the interaction between proteins composing tight junction in premature fetal rabbits.