PENGEMBANGAN METODE DETEKSI KERUSAKAN DNA SPERMATOZOA MENGGUNAKAN PELISIS MEMBRAN DAN PEWARNAAN SEL SPERMATOZOA UNTUK PENINGKATAN KUALITAS SEMEN BEKU SAPI

INTISARI

Teguh Ari Prabowo
20/468223/SPT/00206

Kualitas spermatozoa yang digunakan memberikan pengaruh yang signifikan terhadap kinerja reproduksi termasuk *Service Perconception* (S/C), *Conception Rate* (CR), *Calving Interval* (CI) dan *Day Open* (DO). Salah satu faktor yang memengaruhi kualitas spermatozoa adalah kerusakan DNA spermatozoa. Tingkat kerusakan DNA spermatozoa dapat menyebabkan rendahnya fertilitas, keguguran dan juga menyebabkan gejala prolong *estrus cycle* pada sapi sehingga akan menyebabkan kinerja reproduksi yang tidak optimal. Tujuan penelitian ini adalah untuk memperoleh metode identifikasi kerusakan kromatin DNA spermatozoa. Penelitian tahap I bertujuan untuk memperoleh bahan dan konsentrasi larutan yang dapat menjebak (*trap*) spermatozoa pada *object glass*. Penelitian ini dilakukan dengan cara larutan *agarose* dan LMP-*agarose* dibuat dengan konsentrasi 0,6%; 0,7%; 0,8% dan 0,9%. Setelah larutan dibuat sebanyak 0,5 mL masing-masing larutan dicampurkan dengan 0,5 mL semen beku yang sebelumnya sudah di *thawing*. Kemudian sampel diberikan larutan pelisis membran dan pewarnaan kromatin DNA spermatozoa. Parameter yang diamati yaitu jumlah spermatozoa terjebak di dalam *agarose* dan LMP-*agarose* dan yang mudah di amati di dalam mikroskop. Penelitian dilakukan pengulangan sebanyak enam kali dengan jumlah sampel 24 sampel semen beku sapi bali. Rancangan penelitian ini menggunakan Rancangan Acak Lengkap pola faktorial. Data yang diperoleh dianalisis menggunakan analisis variansi (ANOVA). Hasil penelitian tahap I menunjukkan bahwa konsentrasi *agarose* 0,7% merupakan konsetrasi yang dapat digunakan untuk menjebak (*trap*) spermatozoa. Penelitian tahap kedua yaitu menentukan jenis larutan pelisis membran (*lysis Solution*). Perlakuan pada tahap ini terdiri dari tiga perlakuan yaitu jenis pelisis membran I 0,4 M Tris, 0,8 M DTT, 1% SDS, 0,05 M EDTA, pH 7,5; jenis pelisis membran II 0,4 M Tris, 2 M NaCl, dan 1% SDS, pH 7,5; jenis pelisis membran III 0,09 M Tris-borat dan 0,002 M EDTA, pH 7,5 dengan pengulangan sebanyak enam kali. Tahap ini menggunakan spermatozoa yang telah diberi hidrogen peroksida (H_2O_2) sebagai kontrol. Parameter yang di amati yaitu berupa gambar visual benang-benang kromatin yang keluar di kepala spermatozoa, semakin terlihat benang-benang kromatin di kepala spermatozoa maka pelisis membran tersebut yang terbaik. Spermatozoa yang rusak kemudian dihitung persentasenya. Data yang diperoleh dirancang menggunakan Rancangan Acak Lengkap dan dianalisis menggunakan analisis variansi (ANOVA). Hasil penelitian tahap II menunjukkan bahwa pelisis membran I yang dapat melisiskan sel membran lebih baik dibandingkan dengan kedua pelisis membran lainnya. Penelitian tahap III menggunakan pewarna eosin (konsentrasi 0,5%, 1% dan 2%) dan pewarna *methylene blue* (konsentrasi 0,5%, 1% dan 2%). Parameter yang diamati yaitu benang-benang kromatin DNA spermatozoa yang keluar pada bagian kepala spermatozoa yang mudah di amati

menggunakan mikroskop. Data yang diperoleh dianalisis secara deskriptif. Hasil penelitian tahap III menunjukkan bahwa konsentrasi eosin 1% dan *methylene blue* 1 % dapat mendeteksi kerusakan DNA spermatozoa. Penelitian tahap IV menguji metode deteksi kerusakan DNA spermatozoa yang dikembangkan dengan kit komersial. Parameter yang di amati yaitu berupa jumlah kromatin DNA spermatozoa yang rusak dapat terdeteksi. Spermatozoa yang rusak kemudian dihitung jumlahnya. Data yang diperoleh dianalisis menggunakan analisis deskriptif yang disajikan dalam rataan dan standar deviasi. Hasil penelitian tahap IV menunjukkan bahwa Sperm–Bos–Halomax® dan Kit Uji dapat mendeteksi kerusakan kromatin DNA spermatozoa dengan rataan persentase kerusakan DNA sapi menggunakan metode Sperm–Bos–Halomax® sebesar 1,69±0,67%, sedangkan pada Kit Uji sebesar 2,00±0,61%. Hasil penelitian pada tahap IV menunjukkan bahwa kit uji deteksi kerusakan DNA spermatozoa yang dikembangkan dapat digunakan untuk mengidentifikasi kerusakan DNA. Kesimpulan penelitian ini adalah kit uji dapat diaplikasikan sebagai salah satu metode deteksi kerusakan DNA spermatozoa sapi.

Kata kunci: *Agarose*, eosin, kerusakan DNA, kromatin, *methylene* blue, pelisis membran sel, protamin, semen beku sapi, spermatozoa, sperm boss halomax.

DEVELOPMENT OF SPERMATOZOA DNA DAMAGE DETECTION METHOD USING MEMBRANE LYSING AND SPERMATOZOA CELL STAINING TO IMPROVE THE QUALITY OF FROZEN BOVINE SEMEN

ABSTRACT

Teguh Ari Prabowo
20/468223/SPT/00206

The quality of the spermatozoa utilized exerts a significant influence on reproductive performance, including service perception (S/C), conception rate (CR), calving interval (CI), and day open (DO). One factor that can impact the quality of spermatozoa is the presence of DNA damage in the spermatozoa. The extent of DNA damage in spermatozoa can result in reduced fertility, increased risk of miscarriage, and prolonged estrus cycles in cows, ultimately leading to poor reproductive performance. The objective of this research was to develop a methodology for the detection and assessment of DNA chromatin damage in spermatozoa. The aim of this study was to obtain a technique for identifying spermatozoa DNA chromatin damage. The objective of phase I research was to discover materials and solutions with certain concentrations that have the capability to immobilize spermatozoa on an object glass surface. This was achieved by preparing solutions of agarose and LMP-agarose at concentrations of 0.6%, 0.7%, 0.8%, and 0.9%. A solution of 0.5 mL was prepared for each solution, which was next combined with 0.5 mL of previously thawed frozen semen. After that, the sample was subjected to a membrane lysing solution and received sperm DNA chromatin staining. The characteristics that were recorded included the quantification of spermatozoa that were immobilized in agarose and LMP-agarose, and were afterwards visualized using a microscope. The investigation was replicated on six replications, with a total sample size of 24 frozen semen samples of Balinese cattle. The study had a completely randomized design involving a factorial pattern. The data acquired were subjected to analysis of variance (ANOVA). The initial findings of the investigation indicated that a concentration of 0.8% agarose showed efficacy in capturing spermatozoa. In the second phase of the study, the objective was to identify the appropriate lysis solution for three distinct treatments. These treatments were referred to as membrane lysis type 1, membrane lysis type 2, and type of membrane lyzer. The lysis solution for membrane lysis type 1 consisted of 0.4 M sTris, 0.8 M DTT, 1% SDS, 50 mM EDTA, with a pH of 7.5. For membrane lysis type 2, the lysis solution comprised 0.4 M Tris, 2 M NaCl, and 1% SDS, with a pH of 7.5. Lastly, the type of membrane lyzer utilized a lysis solution containing 0.09 M Tris-borate, and 0.002 M EDTA, with a pH of 7.5. Each treatment was repeated six times to ensure the reliability and accuracy of the results. In this experimental phase, the spermatozoa employed have been subjected to hydrogen peroxide (H_2O_2) as a means of control. The criteria that were assessed consisted of a visual representation depicting chromatin threads originating from the head of the spermatozoa. It was noted that the degree of visibility of these chromatin threads within the spermatozoa head directly correlated with the effectiveness of the membrane lyzer. The number of damaged spermatozoa was then quantified at a magnification of ten times the field of vision. The data was collected using a complete randomised design and

afterwards subjected to analysis of variance (ANOVA). The findings from the subsequent stage of the investigation revealed that the first membrane lyzer exhibited superior efficacy in cell membrane lysis compared to the other two membrane lyzers. The phase III study employed eosin dye at concentrations of 0.5%, 1%, and 2%, as well as methylene blue dye at concentrations of 0.5%, 1%, and 2%. The criteria that were observed in this study were the DNA chromatin threads of the spermatozoa, which were clearly visible at the head of the spermatozoa and easily observable using a binocular microscope. The acquired data were subjected to descriptive analysis. The findings from the third phase of the investigation indicated that the use of 1% eosin and 1% methylene blue concentrations proved effective in identifying DNA damage in spermatozoa. The Phase IV study conducted by evaluating the method for detecting DNA damage in spermatozoa, which was established using a commercially available kit. The characteristics that were observed included the quantification of DNA chromatin damage in spermatozoa. The spermatozoa that had incurred damage were subsequently quantified at a rate of 10 times per field of vision. The data obtained were analysed using descriptive analysis presented as mean and standard deviation. The results of stage IV research show that Sperm-Bos-Halomax® and the Test Kit can detect spermatozoa DNA chromatin damage with an average percentage of bovine DNA damage using the Sperm-Bos-Halomax® method of 1.69 ± 0.67%, while the Test Kit is 2.00 ± 0.61%. The results of research in stage IV show that the developed spermatozoa DNA damage detection test kit can be used to identify DNA damage. The conclusion of this study is that the test kit can be applied as a method of detecting bovine spermatozoa DNA damage.

Keywords: Agarose, eosin, DNA damage, chromatin, methylene blue, cell membrane lyzer, protamine, bovine frozen semen, spermatozoa, sperm–bos–halomax.