Characteristic Several Level of Bovine Serum Albumin (BSA) and Its Combination as Albumin Column for Sperm Sexing

(Karakteristik Berbagai Konsentrasi Bovine Serum Albumin (BSA) dan Kombinasinya sebagai Kolom Albumin untuk Media Sexing Sperma)

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ABSTRACT

The aims of the research were to identify the characteristics of bovine serum albumin (BSA) such as pH, density and viscosity at various concentration and to find out the combination of BSA concentration that quantify as sexing medium. This research used four combination of BSA concentration in the tube as upper and bottom layer consist of 3-6, 4-8, 5-10, and 6-12%, and identified for its characteristics such as pH, viscosity and density, and repeated three times. The data were analysed descriptively. The result showed that pH from these several BSA concentration range between 7.30-7.57. It showed that the pH in the normal range. The value of viscosity and density increased in line with increasing of BSA concentration. The result showed that the BSA combination of 5% at upper layer and 10% at bottom layer resulted almost
the same pH value than others combination, so it will support the sperm quality and viability. In conclusion, characteristic of pH, viscosity, and density of several BSA concentration (3, 4, 5, 6, 8, 10, and 12%) were at normal value and the combination of BSA concentration that more quantify as sexing medium were 5% at upper fraction and 10% at bottom layer.

**Key words:** Bovine serum albumin, sperm sexing, sperm quality

### INTRODUCTION

Serum albumin is the most abundant plasma protein in mammals and multifunctional protein with extraordinary ligand binding capacity, making it a transporter molecule for a diverse range of metabolites, drugs, nutrients, metals and other molecules.

Serum albumin can largely prevent the dilution effect, and it has been assumed that this protein substitutes for vital seminal plasma components. The phenomenon of dilution effect is an event where there is a rapid decrease in motility and an increase in proportion of sperm death when semen is diluted in simple artificial media. Albumin does not act as a protective agent but rather stimulates the motility of dilute sperm suspensions in a reversible fashion. The "protection" afforded by serum albumin against a dilution effect is due to its ability both to stimulate sperm motility and to prevent the cells sticking to the container surface. Sticking-to-glass is potentially damaging to spermatozoa in that detachment of the stuck cells by agitation may physically tear the plasmalemma. Under such circumstances, albumin could be exerting an indirect protective action by preventing sticking.

Bovine Serum Albumin was more effective (on a weight basis) than other commercially available macromolecular substances tested, particularly at maintaining stimulated motility levels, and its action appeared independent of ionic strength and of common constituents of media.

Base on its function, BSA has been used for sperm sexing media especially for bull semen by making many fraction with different concentration of BSA in the tube. The using of BSA for sperm sexing in ram still limited and also the research about combination of BSA concentration still limited. On the other hand, research about ram’s semen processing had been reported such as level of cryoprotectan (Solihati et al. 2018a), addition of antioxidant (Solihati et al 2018b) and about natural proportion of ram’s X-Y sperm (Solihati et al. 2017). The aim of the research were to identify the characteristic of bovine serum albumin (BSA) such as pH, density and viscosity at various concentration of BSA as albumin column for sperm sexing.
MATERIAL AND METHOD

The object of research was BSA dissolved in media BO with concentrations of 3, 4, 5, 6, 8, 10, and 12%. Bovine Serum Albumin with various concentrations identified its characteristics consisting of pH, density and viscosity. Testing these characteristics was repeated three times and then averaged.

Evaluation of pH characteristic

There were 3 steps that need to be done in measuring pH: (a) Provide electrode rods, buffer solutions and samples to be measured, (b) Calibration by measuring the content of electrolyte cells 2 standard buffer solutions, (c) Measuring electrolyte cells from the sample used (Emerson Process Management 2010).

pH is a parameter that can be measured and the tool used to measure the pH of a solution is called a pH meter. The most important component of the pH meter is the probe or electrode rod connected to the electrometer and pH reader. The pH meter device was calibrated using a buffer solution known hydrogen ion levels (Karastogianni et al. 2016). Determination of the pH value of the BSA column can be done with the following steps: Prepare 7 measuring cups, each measuring cup is inserted 5 ml BSA solution with various combinations of concentrations; Prepare 50 ml of pH buffer and 50 ml of Aquades in a measuring cup; Enter the pH meter into distilled water; Enter the pH meter into the pH buffer, until the pH meter reaches 7 (neutral). Then the pH meter was inserted into a measuring cup containing BSA, neglected 3 times and averages were calculated. Repeat for 6 measuring cups containing the next BSA solution.

Evaluation of viscosity characteristic

This method is determined by Poisulle’s law using the Ostwold viscometer. Determination is done by measuring the time needed to drain the liquid in the capillary pipe from a sign to b. The liquid measured by the viscosity is inserted into the viscometer which is placed on the thermostat. The liquid is then sucked with a pump into the ball until above the sign a. The liquid is left to run down and the time needed from a to b is recorded using a stopwatch (Rosiana 2005). Ostwold viscosimeter works based on the time interval required by a certain amount of solution to flow through the capillary tube by the force caused by the weight of the solution itself (Jati & Rizkiana 2015). If η and the comparison liquid are known, then measuring the time needed to megaliri both fluids through the same tool can be determined η liquid known to be tight. Comparative fluid commonly used was water (Sutiah et al. 2008).
Determining the value of viscosity can be done by the steps as follows: Add 10 ml of BSA solution with a concentration of 3% to the ostwald viscometer; Suction until the sign a (top line); Let it flow until the sign b (underline); Record the flow time; Repeat up to 3 times.

Calculate the viscosity with the formula as follows:
\[ \eta = \eta_0 \frac{(t \cdot \rho)}{(t_0 \cdot \rho_0)} \]

\( \eta \) = Viscosity of sample liquid
\( \eta_0 \) = Viscosity of the comparison liquid
\( t \) = Time of liquid sample flow
\( t_0 \) = Time of comparison flow
\( \rho \) = Density of sample flow
\( \rho_0 \) = Comparative flow density

Repeat the above treatment for BSA solutions concentrating 4, 5, 6%, 8, 10, and 12%.

Evaluation of density characteristic

The density of the BSA solution can be tested by the picnometer. Following were the steps to find out the density value: Blank picnometer weight (\( m_0 \)); Enter 5 ml of BSA solution with a concentration of 3% to the limit indicated by the pycnometer; Weigh the pycnometer which has been filled with sample solution (\( m_1 \)); Record the results and record the temperature of the solution when the pycnometer is weighed.

Calculate the density of the solution with the following formula:
\[ \rho = \frac{(m_1-m_0)}{V_p} \]

\( \rho \) = Density
\( m_1 \) = Pycnometer mass and sample
\( m_0 \) = Pycnometer mass is empty
\( V_p \) = Volume of sample in pycnometer

All treatment repeat for BSA solutions concentrating 4, 5, 6, 8, 10, and 12%.

RESULT AND DISCUSSION

Characteristic of pH, density and viscosity of bovine serum albumin

Identification was carried out on pH, density, and viscosity values of each BSA concentration used for the process of sexing semen. The average results of pH, density and viscosity of BSA at various concentrations are listed in Table 1. It showed the pH of BSA concentration of 3% (7.33), 4% (7.37), 5% (7.43), 6% (7.50), 8% (7.57), 10% (7.40), and 12% (7.30). The pH value in this study ranged from 7.33-7.57, which means that the BSA solution was in a neutral condition. pH is a measure of the concentration of hydrogen ions (H\(^+\)) and hydroxide (-OH) in a
solution. This hydrogen ion concentration can determine the acidity or basicity of a solution. Acid and base dissolved in water can change the concentration of H+ and -OH in solution. Acid increases the concentration of hydrogen ions because the relative amounts of H+ and -OH ions in solution must remain balanced, the acid decreases the concentration of -OH ions and vice versa for alkaline substances (Emerson Process Management 2010).

Bovine Serume Albumin contains a simple protein in the form of amino acids. Nurhidayah (2013) states that proteins containing amino acids are hydrophilic, namely proteins whose amino acid residues are water-like. This is due to the presence of a hydrogen group in the peptide which is a polar organic molecule, so it will form water in the presence of an OH group. Each BSA concentration has a different pH, because there are more substances added to higher concentrations. According to Imelda (2007) changes in pH values are influenced by storage time and the difference in the addition of substances in solution.

Density is a physical property that describes the bond density of the constituent materials of a material. The density level is influenced by the type, amount, percentage of material and fluid. Density is the density of substances in filling space. According to Giancoli (2001) density can be interpreted as a measure or distance between particles in a substance. Density can be interpreted as the size or distance between particles in a substance. The density in the fluid represented by rho (ρ) is defined as the density which is interpreted as the mass of fluid per unit volume. The value of density of a substance is influenced by temperature, the higher the temperature, the value of density will be lower, because the bonds that exist in the molecule will be released (Giancoli 2001).

Bovine Serum Albumin density at some BSA concentrations was 3% (1.0502 gr/ml), 4% (1.0524 g/ml), 5% (1.0547 g/ml), 6% (1.0574 g/ml), 8% (1.0620 g/ml), 10% (1.0661 g/ml), 12% (1.0720 g/ml). The higher the concentration of BSA, the more molecules contained in it, so the value of density was greater. Another factor that affected the density was temperature. According to Giancoli (2001) the density value of a substance is influenced by temperature, the higher the temperature, the lower the value of density, because the bonds in the molecule will be released.

Viscosity is a value that states the flow rate of a substance. The viscosity of a protein solution is influenced by the internal characteristics of the solution such as molecular weight, size, volume, shape and surface density. In addition, viscosity is also influenced by environmental factors such as pH, temperature, ion strength, ion type, and heating (Friedli 2006). Viscosity is a measure of the resistance (resistance) of a fluid to flow. The greater the viscosity the slower the flow of fluid (Chang 2005). The viscosity value can be expressed in centipoise (cP) (Kristine et al. 2008).

Bovine Serum Albumin viscosity increased with increasing concentration, namely at a concentration of 3% (0.7996 cP), 4% (0.8246 cP), 5% (0.8641 cP), 6% (0.9054 cP), 8% (0.9606 cP), 10% (1.0378 cP), 12% (1.1638 cP). Higher concentrations
of BSA will have greater viscosity. This condition occurred because higher concentrations would be thicker, so the time needed to flow in the solution was longer. This was in accordance with the statement of Jati & Rizkiana (2015) that the longer the velocity of solution flow time, the greater the value of viscosity. According to Donal (2004) the viscosity of a liquid will decrease with increasing temperature. This is related to the molecular structure of liquid. Liquid molecules are in close proximity to strong cohesion forces between molecules and barriers to relative motion between fluid layers that are related to the forces between these molecules. With increasing temperature this cohesion force decreases and results in reduced movement resistance.

In theory the value of the viscosity of a liquid will decrease with increasing temperature. This is related to the molecular structure of liquid. Liquid molecules are in close proximity to strong cohesion forces between molecules and barriers to relative motion between adjacent fluid layers that are related to the forces between these molecules. With increasing temperature, this cohesion style decreases and results in reduced movement barriers (Donal 2004).

**Table 1. Characteristic of pH, density and viscosity of Bovine Serum Albumin**

<table>
<thead>
<tr>
<th>BSA Concentration (%)</th>
<th>pH</th>
<th>Density (g/ml)</th>
<th>Viscosity (cP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>7.33±0.06</td>
<td>1.0502±0.0010</td>
<td>0.7996±0.0031</td>
</tr>
<tr>
<td>4</td>
<td>7.37±0.06</td>
<td>1.0524±0.0002</td>
<td>0.8246±0.0018</td>
</tr>
<tr>
<td>5</td>
<td>7.43±0.06</td>
<td>1.0547±0.0002</td>
<td>0.8641±0.0011</td>
</tr>
<tr>
<td>6</td>
<td>7.50±0.10</td>
<td>1.0574±0.0003</td>
<td>0.9054±0.0051</td>
</tr>
<tr>
<td>8</td>
<td>7.57±0.15</td>
<td>1.0620±0.0002</td>
<td>0.9606±0.0042</td>
</tr>
<tr>
<td>10</td>
<td>7.40±0.10</td>
<td>1.0661±0.0002</td>
<td>1.0378±0.0098</td>
</tr>
<tr>
<td>12</td>
<td>7.30±0.00</td>
<td>1.0720±0.0001</td>
<td>1.1638±0.0058</td>
</tr>
</tbody>
</table>

**Characteristic combination of bovine serum albumin concentration**

Characteristic combination of several BSA concentration was summarized in Table 2. Based on Table 2 the value of pH from BSA concentration combination of 3-6% and 4-8% showed pH value at upper layer was lower than bottom layer, but pH value from 6-12% were higher at upper layer than bottom layer. We found that pH value from 5% and 10% BSA concentration was surprisingly already the same. Concentration combination of 5-10% showed the value both upper and bottom layer. It would be the advantage if we used these combination for sperm sexing, whereas there was no difference pH condition in sexing media. This pH value will give comfort condition for sperm through sexing treatment with albumin columns, because sperm were not pass internal pH changes. Regarding to Contri et al. (2013), the sperm movement was significantly influenced by pH of medium. Up
and down the external pH would modify internal pH that regulate sperm motility that linked with mitochondria activity. Structure and function of mitochondria clearly effect to sperm motility. The enzyme at mitochondria active at neutral pH. Decreasing of sperm pH will decreasing sperm motility.

It could be showed that density value from combination of BSA concentration used for sexing media were different. Based on Table 2 it showed differences of density value from BSA concentration between bottom fraction and upper fraktion. Density of bottom fraction was higher than upper fraction.

It also could be showed that viscosity value from combination of BSA concentration were different. Base on Table 2, it showed differences viscosity value from BSA concentration between bottom fraction and upper fraction. Viscosity of bottom fraction was higher than upper fraction.

Based on the evaluation of these density and viscosity, it showed that density and viscosity of bottom fraction were higher than upper fraction. It showed that the sperm needed more energy to passed the bottom layer, so there were fewer sperm at bottom fraction. Solihati et al. (2008) stated that higher concentration of BSA at bottom fraction would more difficult to sperm passed the BSA layer, so we found fewer sperm on bottom layer.

### Table 2. Characteristic combination of bovine serum albumin concentration

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Combination consentration of BSA at upper/bottom fraction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>3/6 4/8 5/10 6/12</td>
</tr>
<tr>
<td>Density (g/ml)</td>
<td>1.0502/1.0574 1.0524/1.0620 1.0547/1.0661 1.0574/1.0720</td>
</tr>
<tr>
<td>Viscosity (cP)</td>
<td>0.7996/0.9054 0.8246/0.9606 0.8641/1.0378 0.9054/1.1638</td>
</tr>
</tbody>
</table>

**CONCLUSION**

Base on the result of this research, it was concluded that characteristic pH, density and viscosity of several BSA concentration were different, the combination of BSA concentration that quantify as sexing medium were 5% at upper fraction and 10% at bottom layer.

**AKNOWLEDGEMENT**

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DAFTAR PUSTAKA


DISCUSSION

Pertanyaan:

1. Apakah ada perbedaan BJ antara kromosom X dan Y, sehingga lebih mudah dipisahkan?

2. Apakah metode BSA ini merupakan metode sexing sperma yang pernah ada, atau metode baru yang ibu temukan? Apakah ada kondisi khusus tertentu yang harus diterapkan di lingkungan tabung reaksi agar sperma tetap optimal dapat bergerak aktif dan berapakah waktu maksimal pengamatan dilakukan hingga sperma yang diamati tidak motil atau non aktif.


Jawaban:

1. Sperma pembawa kromosom X dan Y memiliki perbedaan dalam hal ukuran luas kepala dan kecepatan, sperma pembawa kromosom X memiliki ukuran kepala lebih besar dibanding X, namun sperma Y memiliki pergerakan lebih cepat. Perbedaan ini yang menjadi dasar dilakukannya sexing sperma.

2. Metode BSA merupakan metode sexing sperma yang sudah pernah ada. Metode sexing dengan BSA yaitu dengan menggunakan tabung yang diisi dua lapisan BSA berbeda konsentrasi dan ditempatkan di dalam incubator 37°C. Lama waktu pengamatan hingga sperma yang diamati tidak motil atau non aktif yaitu sekitar 200 jam.

3. Lapisan BSA yang umum digunakan yaitu dua lapisan, lapisan atas dan lapisan bawah. pH penting untuk sexing karena berpengaruh terhadap aktivitas mitokondria, dimana enzim-enzim di mitokondria akan aktif pada pH normal, sehingga kondisi pH antara lapisan atas dan lapisan bawah yang hampir sama akan membuat aktivitas mitokondria berjalan dengan normal dan motilitas akan terjaga. Viskositas berkaitan dengan kemampuan sperma untuk menembus lapisan BSA, sehingga diharapkan sperma pembawa kromosom Y yang memiliki motilitas tinggi dapat terkonsentrasi di lapisan bawah.