Boer Spermatozoa Quality in Different Incubation Periods and Medium for In Vitro Fertilization (IVF) Preparation

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Abstract. One of the developments in biotechnology is in vitro fertilization (IVF) requires a preparation process, one of it is spermatozoa. Preparation of spermatozoa is a stage of purification and separation as well as to eliminate bacteria and clear the unwanted residues. In preparation usually use a special medium to maintain the quality, as energy sources, and others. This research aims to determine the effect of incubation time on the quality of spermatozoa of Boer goats in two different types of medium, Tissue Culture Medium (TCM) -199 and Phosphate Buffer Saline (PBS). This research used 13 frozen semen obtained from Balai Besar Inseminasi Buatan (BBIB) Singosari then analysed the standard quality of post-thawing spermatozoa for next stages treatment. Post-thawing semen then prepared and tested in two types of media, there are TCM-199 and PBS. In each media, Semen was incubated at 38°C CO2 5% for 30 (P1), 45 (P2), and 60 (P3) minutes. The results of the study then observed sperm quality, like; motility, viability, abnormality, and concentration, in addition to quality, the effect of incubation time was analyzed using Randomized Block Design (RBD) and continued with further testing of the Least Significance Different (LSD). The results showed the average sperm motility for TCM-199 medium P1, P2, and P3 respectively; 28% ± 10.95, 34% ± 8.94, and 26% ± 5.48, while for PBS P1, P2, and P3 medium respectively; 58% ± 4.47, 56% ± 8.94, and 46% ± 8.94. The conclusion of this study is that PBS medium is better for maintaining sperm quality while incubation, especially in the 30 minute compared to TCM-199 medium.

Keywords: Boer Goat, Spermatozoa, TCM-199, PBS, IVF

1. Introduction
Cryopreservation in semen contribute the damages of spermatozoa like oxidative stress, ice crystal formation, osmotic changes cold shock, and cell membrane lipid–protein reorganizations. Then, it is needed to preparation the best sperm for next appliance like In vitro fertilization (IVF), artificial insemination, etc. IVF now-days is commonly used to increasing the chance of successful fertilization. Despite the complexity of spermatozoa structure, it is needed to prepare and maintain the spermatozoa quality. An efficient sperm preparation technique used to retrieval of high quality spermatozoa can contributes the good embryos development with high implantation potential [1]. An understanding of the function of various sperm molecules in the process of in vivo fertilization will allow new strategies
to regulate these events and alter sperm function and male fertility. The sperm quality could be significantly improved by enhancing progressive motility and increasing the number of morphologically normal spermatozoa [2].

One method of preparation to improve the sperm quality is washing procedures. This washing technique has been widely applied, but in this case, for appliance using Boer breed goat spermatozoa has not been studied. Washing method functions are to separating the healthy, motile sperm from unwanted materials like enzyme, proteins, fluids, bacteria, hormones, and also dead sperm cells. Washing methods may be an important step for improving the quality of spermatozoa by reducing the formation of free oxygen radicals (ROS) after sperm preparation and decreasing the release of lymphokines or cytokines [3]. Sperm quality and fertility needs to be improved. Some normal spermatozoa are lost during the selection process but overall the advantages of improved longevity and fertility in the selected spermatozoa outweigh the disadvantages. In this research, our objective was to compare the incubation time on the quality of spermatozoa of Boer goats in two different types of medium, Tissue Culture Medium (TCM) -199 and Phosphate Buffer Saline (PBS).

2. Material and Method

Thirteen straws of frozen semen from Boer goat semen with post thawing motility (PTM) >40% and viability >80% divided into 2 treatments, medium addition of Tissue Culture Medium (TCM)-199 and Phosphate Buffer Saline (PBS). Furthermore, the semen using single washed with 1500 rpm centrifugation for 5 minutes with the treatment medium. Supernatant removed and left for ± 1ml. Furthermore, each treatment added TCM-199 and PBS as much as 1 ml to the tube and incubated for at 37°C for 30 minutes (P1), 45 minutes (P2) and 60 minutes (P3) in each treatment was replicated five times.

Quality analysis of post-incubation spermatozoa is done by standard motility test, viability test, and abnormality test. The sperm motility was determined by microscope for ×400 magnifying. The percentage of motile spermatozoa were assessed with a scale of 0-100%. Sperm concentrations of were determined using hemocytometer (Neubauer Improved, Marienfeld, Germany). Then the data analysis is continued by using Randomized Block Design (RBD) and continued with the Least Significant Difference test (LSD) to determine the level of difference.

3. Result and Discussion

3.1. Semen quality of post-thawing

The results of post-thawing semen quality analysis (table 1) show the average mass motility of 3+ and individual motility of 57.5 ± 7.43%. Viabilities on spermatozoa of 64.5 ± 14.94% showed that the semen was well categorized to be used for IVF. Abnormalities of 3.88 ± 2.59% which shows the normal percentage, because the normality of spermatozoa is still above 80% [4] is categorized as suitable for use for insemination or IVF. Thawing the semen after being cryopreserved is known to disrupt the plasma membrane of spermatozoa and it is also induces the premature capacitation of a spermatozoa [5], and because of that, the preparation like washing needs to be applied very carefully to maintain the best sperm left.

| No | Parameter                        | Mean±SD       |
|----|----------------------------------|---------------|
| 1  | Mass motility                    | 3+            |
| 2  | Individual motility (%)          | 57.5 ±7.43    |
| 3  | Viability (%)                    | 64.5 ±14.94   |
| 4  | Abnormality (%)                  | 3.88 ±2.59    |
| 5  | Concentration (mill/ml)          | 38.53×10⁶ ± 7.75 |
3.2. Semen quality after incubation

The results of semen quality analysis after incubation are shown in table 2. The highest motility of spermatozoa is shown in the treatment of P1 with PBS medium. The average of sperm motility using TCM-199 is below 34% while with PBS medium it is still above 46%, it means phosphate buffer saline as a medium is able to maintain spermatozoa motility because the pH tends to be more stable when compared to TCM-199 medium which have more complexity component to keep the stability. The highest viability of spermatozoa was known in the P3 treatment using PBS medium, but this increase was not directly proportional to the length of treatment, because in the P1 treatment, it was higher than the P2 treatment in the PBS medium. Whereas on TCM-199 medium, the average is still under treatment with PBS medium, which is below 52%.

The effect after incubation treatment from frozen spermatozoa which is can returns the ability of normal spermatozoa cell in right temperature. Sperm preparation are important to remove unwanted molecules and anti-genic proteins so the viability of sperm cell can be maintained [6]. Viability percentage of more than 50% in PBS medium after incubation shows that the right temperature and medium prolong the life of sperm cell.

Table 2. Semen quality after incubation

| Parameters       | Medium     | P1          | P2          | P3          |
|------------------|------------|-------------|-------------|-------------|
| Motility (%)     | TCM-199    | 28 ± 8.66   | 34 ± 10.48* | 26 ± 5.48   |
|                  | PBS        | 58 ± 4.47   | 56 ± 8.94   | 46 ± 14.66* |
| Viability (%)    | TCM-199    | 52.7 ± 28.07| 31.3 ± 24.75*| 52.6 ± 28.11|
|                  | PBS        | 63.7 ± 11.71| 60 ± 15.88  | 74.4 ± 6.85 |
| Abnormality (%)  | TCM-199    | 4.6 ± 2.07  | 7.6 ± 4.28  | 6.8 ± 3.11  |
|                  | PBS        | 6.6 ± 4.88  | 4.4 ± 3.78  | 3.2 ± 3.08  |
| Concentration (mill/ml) | TCM-199    | 11.8 ± 3.19*| 10.2 ± 1.79 | 10.4 ± 3.21 |
|                  | PBS        | 13.2 ± 5.54*| 32.2 ± 11.48| 21 ± 9.11   |

Note: *shows significant difference from same row (P<0.01)

Percentage of spermatozoa abnormalities showed that there were no significant differences in each treatment medium also from PTM. The increase in abnormalities that occur due to a series of treatments starting from the thawing process after being cryopreserved [7], the addition of the medium, and centrifugation stages. Abnormalities also can occur in the phase of spermatogenesis, exits, until ejaculation occurs. Spermatozoa concentration results of the analysis showed the use of TCM-199 medium was lower than the use of PBS medium. The highest concentration was in P2 treatment using PBS medium, 32.2 ± 11.48 million / ml. The medium used affects the adaptability of spermatozoa. There is also interaction between length of incubation and sperm concentration on individual motility of spermatozoa, the higher sperm concentration and the longer incubation also affect the motility [8].

4. Conclusion

In conclusion, sperm washing method the best in this research is using PBS medium is better for maintaining sperm quality while incubation, especially in the 30 minute compared using to TCM-199 medium. This study requires further and deeper analysis and repetition, especially on the incubation period.

5. References

[1] Jiang M, Wen Y, Yang W, He W, Zhang B, Cai L 2016 *IJE*P*9*(8) 8550-8554
[2] Samardzija S, Getz I, Lojkic M, Valpotic H, Djuricic H 2015 *SOJ Veterinary Sciences* 1(2) 1-7
[3] Morrell J M, Kumaresan A, Johannisson A 2017 Proceedings of the 31st Annual Meeting of the Brazilian Embryo Technology Society (SBTE) Cabo de Santo Agostinho: Brazil

[4] Chaveiro A, Cerqueira C, Silva J, Franco J and Moreira da-Silva 2015 Iranian Journal of Veterinary Research 16(2) 188-193

[5] Ciptadi G, Ihsan M N, Rahayu S, Wajuhningsih S, Muzanaroh A, Chotimah C, Ardyah I P, Putra R P 2017 Research Journal of Life Science 4(3) 1-7

[6] Turhan N, Pekel A, Onaran Y, İltemir-Duvan Z C, Bayrak O 2011 Turkey Journal Medicinal Sciences 41 (1) 39-44

[7] Rozati H, Handley T and Jayasena C N 2017 Journal of clinical medicine 6(9) 89-95

[8] Sri W, Hermanto, Nuryadi, Agus B, Panji B 2012 Engineering and Technology International Journal of Animal and Veterinary Sciences 6(12) 6-12