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by Erma Safitri

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TrilasSardjito, PudjiSrianto, Chairul Anwar Nidom, Imam Mustofa and Erma Safitri[1]

Department of Reproduction Veteriner, Faculty of Veterinary Medicine, UniversitasAirlangga, Surabaya, Indonesia, 60115

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Abstract

Artificial insemination with frozen semen of Limousin bulls was evaluated for the post thawing changes in quality characteristics in semen and their effect on the sperm viability, motility and spermatozoa membrane integrity. Besides the frost-bonding effect on the production of heat shock protein (HSP) and their protecting nature against the extreme temperature changes on the extra and intracellular integrity of spermatozoa were evaluated. Twenty four semen samples randomly drawn were subjected to freezing as per regulation of the Director General of Animal Husbandry, Indonesia. The frozen semen after thawing showed decreasing viability from 89.67±4.03% to 60.00±3.30%; motility from 76.87±4.90% to 50.42±4.87% and sperm membrane integrity from 65.88±5.82% to 29.00±3.55%. These values were in accordance with that of minimum percentage of motility of 40 per cent.

Key words : Frozen semen, thawing quality, Limousin bulls.

Beef cattle breeding favours artificial insemination with semen from superior bulls to produce off springs which can attain higher weight at marketing age (Sutarnoand Setyawan, 2015). The production of frozen semen can trigger changes in spermatozoa both in cellular and molecular level. One such problem that might occur during semen freezing is cold-shock resulting in ice crystal formations, electrolyte soluble materials accumulation in cells, which could damage the intracellular segment of spermatozoa. This effect could cause the structural changes in the amino acid of spermatozoa, which again affects the sperm viability (Hafez and Hafez, 2013). The effect of frost-bonding process instigates the protein denaturation and increase the production of heat shock protein (HSP). The use of ethylene glycol and sucrose can protect cells from cold shock and increase the production of HSP70, a variant of heat shock protein. This will protect the damaged cells from extreme temperature fluctuations. The damages during thawing process can affect the sperm viability, motility, membrane integrity and the fertility per cent fertile of spermatozoa. Hence the study of freezing the Baluran bull semen was conducted to assess their effect on the above characteristics.

Materials and Methods

Limousin bulls (Baluran) was utilized in the study of post freezing analysis on the changes in the semen quality parameters. The study was conducted at the frost-bonding semen laboratory BIB Faculty of Veterinary Medicine located at Taman TernakPendidikanUniversitasAirlangga, Gresik, Indonesia.

The freezing of Baluran bull semen was carried out as per the standard operational procedure (SOP) of the Director General of Animal Husbandry of Republic Indonesia (2007) guidelines for production and distribution of frozen semen.

The examination of HSP70 was done to calculate the total spermatozoa possessing positive immunoreaction as per (Fuchs and Auer, 2010). Immunoreactive spermatozoa will initiate production of coloured chromogen which changes from brown to blackish, while negative immunoreative cells will not exhibit colour reaction. The examination was conducted with luminescence microscope Nikon H600L. Twenty four frozen semen samples, randomly drawn were used for the quality assessment in comparison with that of fresh semen to assess the changes that might occur during the freezing and thawing process.

[1]*Corresponding author* : Email : rma_fispro@yahoo.com

Table I. The quality characterization of BaluranLimousin bulls fresh semen and thawed frozen semen and their HSP70 immunoreactivity percentage.

	Quality parameters			
Semen samples	**Viability**	**Motility**	**Membrane Integrity**	**Immunocytochemical protein HSP70**
Fresh	89.67±4.03	76.87±4.90	65.88±5.82	10
Frozen	60.00± 3.30	50.42±4.87	29.00±3.55	30

Results and Discussion

The semen quality changes are presented in the Table I. There was a decrease in the per cent viability from 89.67 to 60.00, motility from 76.87 to 50.42 and integrity of spermatozoa membrane from 65.88 to 29.00. The post thawing motility per cent was 50.42 which is considered as acceptable as minimum sperm motility standard (Komariah *et al*. 2013).

There was an increase of HSP70 extraction percentage in frozen semen group as much as 30 percent compared to fresh semen group which showed only 10 percent (Table I). Spermatozoa which showed positive immuno reactive extraction against HSP70 was coloured brown (Fuchs and Auer, *loc. cit*) in the head and neck region of the spermatozoa.

Aminasari, (2009) has reported that the post thawing sperm motility in bulls aged 3,8,9 and 11 years were 47.8±1.8; 43.8±2.1; 47.0±2.3 and 46.8±1.2% which is in agreement with the observed recorded motility % in the present study.

The semen diluents are used to prolong the quality characteristics of sperm (viability, motility and membrane integrity) during freezing or dilution process (Paulenzet *et al*., 2002). Membrane damages initiated the action of extrinsic factors against the DNA leading to possible DNA fragmentation (Hafez and Hafez, *loc. cit*).

The cryoprotectant is expected to protect spermatozoa from cold shock and increase the production of HSP70, which retain the biochemical activity to restore protein which may undergo misfolding, unfolding or abnormaly folded protein, synthesis of protein, transport and translocation and prevent their aggregation, (Zhang *et al*., 2016). Hence, the presence of HSP70 is considered as the indicator of spermatozoa protection during the freezing process.

The freezing process causes stress to the sperm cell resulting in triggering changes in the protein structure and its role inside the cells. Several studies reported that a group of protein in micromolecular size known as heat stress protein or widely known as heat shock protein (HSP) which played an active role in the process of cells to protect the spermatozoa (Kacimi *et al*., 2000). The results of this study showed that there is an increase in the production processing of HSP (HSP70) in frozen semen.

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