

**ABSTRACT****The role reactive oxygen species in mechanism of damage membrane integrity of swamp buffalo sperm after separation by percoll gradient density centrifugation****Study Experiment Laboratoric****Dasrul**

The objective of this research was to find out the effect of separation of swamp buffalo sperm by using percoll gradient density centrifugation on the the accumulation of reactive oxygen species (ROS) and the role in mechanism of damage of integrity membrane of sperm

The research is a laboratory study using a true experimental design, used swamp buffalo of sperm divided into 3 groups i.e (no centrifugation/ control; percoll density gradient centrifugation for 5 minutes and percoll density gradient centrifugation for 10 minutes). Measurement were conducted on the level of ROS accumulation, level of malondialdehyde (MDA), of docoxahexanoic acid (DHA) and percentage intact plasma membrane. The collected data in this research were analysed with analysis varians, regression dan path analysis

These result showed that separation of sperm by percoll gradient density centrifugation could increase of ROS accumulation and the level of MDA significantly ( $p < 0.01$ ), while the level of DHA membrane and percentage intact plasma membrane sperm decrease significantly ( $p < 0,01$ ). The increase of ROS accumulation, level of MDA and decrease of DHA and persentage of intact plasma membrane were significantly higher on after centrifugation gradient percoll density for 10 minutes than percoll gradien density centrifugation for 5 minutes.

The path analysis of ROS accumulation, level of MDA and level of DHA membrane influeced toward the percentage of membrane plasma intact of sperm. The of percentage intact plasma membran influenced by the level of MDA and level of DHA membrane significantly ( $p < 0,01$ ). The level of MDA and level of DHA membrane was influenced by level of ROS accumulation significantly ( $p < 0,01$ ).

It can be concluded that sperm separation of percoll density gradient centrifugation increase of ROS accumulation, level of MDA, decrease of DHA membrane and persentages of intact plasma membran. The of ROS accumulation has effect to the damage of membran plasma integrity by increase of lipid peroxydation and decrease of DHA membran of sperm.

**Key words** : sperm of swamp buffalo, percoll gradient density centrifugation, ROS, MDA, DHA membrane of sperm and intact plasma membrane