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Storage of Bull and Boar Semen : Novel Concepts Derived Using Magnetized Water and Antioxidants

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ABSTRACT

Artificial insemination technique has been contributed immensely for production of livestock worldwide as a critical assisted reproductive technique to preserve and propagate excellent genes in domestic animal industry. In the past decade, methods for semen preservation have been improved mostly in liquid preservation method for boar semen and freezing method for bull semen. Among many factors affecting semen quality during preservation, reactive oxygen species, produced by aerobic respiration in sperm for survival and motility, are unfavorable to sperm physiology. In mammalian cell as well as in the sperm, antioxidant system plays a role in degradation of reactive oxygen species. Magnetized water forms smaller stabilizing water clusters, resulting in high absorption and permeability of the cell for water, implicating its application for semen preservation. Therefore, this review focuses on preservation methods of boar and bull semen with respect to improvement of extender and reduction of reactive oxygen species by using magnetized water and supplementation of antioxidants.

(Key words : Semen preservation, Reactive oxygen species, Antioxidant, Magnetized water)

INTRODUCTION

In the domestic animal industry, the numerous of domestic animal semen were used to production of little using the artificial insemination (A. I.) technique. The liquid storage and cryopreservation of domestic animals semen were caused by improvement of animal industry. For decades, semen storage were consistently studied by many researcher. Moreover, storage technique of domestic animal semen were widely effect to improvement of A. I. technique, animal breeding, increasing of little production and improvement in farm household economy (Roca *et al*., 2006).

Because the sperm cannot cell division comparison normal somatic cell, the sperm were very sensitive of physical damage, chemical damage and temperature. Especially, variation of temperature were influenced to sperm viability that effect of high temperature on motility was quick in action. The influence of sperm on low temperature and cooling were decreasing motility, damage of plasma membrane, acrosome membrane mitochondrial membrane, DNA and increasing of apoptosis factor (Boe-Hansen *et al*., 2005; Johnson *et al*., 2000; Lopez Rodriguez *et al*., 2012; Shimatsu *et al*., 2002). This phenomenon were indicated that sperm were sensitivly tolerance with low temperature (Maxwell and Johnson, 1997). Damage of sperm were caused to decrease viability, motility and destruction of sperm organelles (Hendricks and Hansen, 2009). Due to the influence were interrupted to sperm-oocyte complex, decreased fertility rate and pregnancy. Ultimately, there were closely connected with low temperature and ability of sperm fertility.

Generally, principle of sperm on liquid preservation and cryopreservation was kept the low activation energy. The effect of low temperature on sperm was maintained low activation energy environment which state on sperm was decreased motility with low energy consumption, semen would keep for a long time. The great advantage of semen storage and A. I. were that genetic ability of boar can be transferred to amount of sows, breeding to genetic improvements (Barbas and Mascarenhas, 2009; Johnson *et al*., 2000).

The mammalian sperm include high levels polyunsaturated fatty acids, and there is immensely weak

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to oxidative stress which is made of reactive oxygen species (ROS; Cassani *et al*., 2005). Also, it has been speculated that ROS which influence sperm physiology (Sanocka and Kurpisz, 2004). The cells are basic unit of all organisms because all organisms are made from cells. All tissues are made up of one or many cells and all organs are composed of one or many tissues. Also, animals made up of many organs. In order to live, the cells produce to energy by metabolism. Above this, water is used for liquid preservatison or cryopreservation of sperm. Therefore, water is very important factor on experiment of liquid preservation and cryopreservation in semen. This review is to introduce storage technique improved in boar and bull semen in extender and ROS aspects that propose preservation of bull and boar semen using magnetized water.

STORAGE OF BOAR SEMEN: LIQUID PRESERVATION

Boar sperm was composed to sperm and amount of seminal plasma in comparison with other mammalian species, which prove sperm for moving to the female reproductive tract such as uterine, oviduct and ovary with the necessary nutrients for metabolic. The ejaculated sperm has high metabolic activity that can be maintained over a limited time, as establishment of preservation of boar sperm have been extensively studied by many researcher. Thus, to preserve spermatozoa for prolonged times, their metabolic activity needs to be reduced by diluting it in suitable extender and lowering the temperature. The time required for the boar to produce a mature sperm possible of fertilization is generally 6~8 weeks. Generally boars are ejaculated for use in A. I. techniques approximately one to half times per week (Johnson *et al*., 2000).

Liquid semen extenders are designed to prolong viability of sperm following ejaculation. Many researched proposed that boar semen extender have to accomplish five basic function: 1) provide nutrients for sperm metabolism; 2) stabilize sperm membranes and prevent capacitation; 3) neutralize metabolic waste products; 4) maintain an osmotic equilibrium; and 5) retard bacterial growth during storage (Flowers, 1997). To accomplish these task, four main components are added to boar semen extenders: 1) energy source (e.g. glucose, lactose and sodium citrate), 2) pH buffering system (e.g. Tris, HEPES, sodium bicarbonate and pH 6.8~7.8), 3) plasma membrane stabilizer (bovine serum albumin, ethylenediamietetraacetic acid) and 4) antibiotic to prevent bacterial growth (e.g. gentamycin, penicillin). Semen extenders are commonly classified as short (3 days) or long (5~7 days) term based on their capacity to maintain viability, acrosome reaction and motility. Characteristic of sperm have been changed to be reduced in preservation of liquid extender. Diluter can be divided into two groups that designed for short-term preservation (less than 1~3 days) which are Beltsville liquid (BL-1), Beltsville thawing solution (BTS), Illinois variable temperature (IVT), Kiev and Vital®. The diluter for long term semen preservation (over 4 days) which are Acromax®, Androhep®, Modena, MR-A®, MULBERRY III®, Reading, X-Cell®, Zorlesco and ZORPVA (Gadea, 2003). General composition for used boar semen extenders which were BTS (Pursel and Johnson, 1975), Zorlesco (Gadea, 2003), IVT (DU MESNIL and Dauzier, 1959), Kiev (Plisko, 1965), Modena (Johnson *et al*., 1988), Androhep (Paulenz *et al*., 2000), MRA (Rodríguez-Gil and Rigau, 1995), ZORPVA (Gadea, 2003) and Reading (Revell and Glossop, 1989) were studied by many researcher.

Bovine serum albumin (BSA) has been closely implicated in ROS activity. BSA is a macromolecule complex protein isolated from bovine plasma. BSA in semen extender is played role to act as a membrane stabilizer (Blank *et al*., 1976). Many research speculated that binding of BSA on sperm may be important for physical properties of sperm required for fusion to the oocytes. Moreover, addition of BSA to preservation media has been shown to maintain motility and prevent lipid peroxidation in rabbit (Alvarez and Storey, 1982). Even if clearly signaling for how BSA prevent lipid peroxidation are not known, many researcher suggest that sin BSA is too large to traverse the plasma membrane, BSA probably protect against lipid peroxidation by to attaching of extracellular side of the plasma membrane. Function of BSA on sperm has been used to preservation of semen that a common additive in the long time extender such as Androhep (Flowers, 1997).

STORAGE OF BULL SEMEN: CRYOPRESERVATION

In cattle, artificial insemination with frozen-thawed sperm is widely used for more than 30 years. Mostly bull semen were caused to damage by exposed external environment such as light, temperature and chemical materials during ejaculation of sperm, dilution processing with semen extender and transportation for cryopreservation from ejaculation place (field or farm) and production of frozen semen location (Kadirvel *et al*., 2009). The ejaculated bull sperm during transportation

were generated to spend rapidly energy with high mitochondrial activity in high temperature condition, influenced to cold shock with low temperature condition (Barbas and Mascarenhas, 2009). A few study were research to develop to semen transport system for cryopreservation and fertility in bull sperm (Lee *et al*., 2013).

Such decrease in utility was maybe complexes of two points of view that were the loss of sperm viability and damage in the potential capability to fertilize (Celeghini *et al*., 2008; Watson, 2000). Although cryopreservation were worthwhile skills for helping endangered animals, it could cause various physical and chemical damages to sperm membrane, outbreak to ROS, oxidative damage to membrane phospholipids and DNA. (Meyers, 2005). Nevertheless, the cryo-damage occurred by freezing processing and preservation can be minimized to use of optimizing cooling rate and cryoprotectant (Jiang *et al*., 2007). In fact, to improve of bull sperm on cryopreservation were accomplished to addition of various antioxidant (Foote *et al*., 2002; Stradaioli *et al*., 2007), regulation of cooling rate and osmotic pressure (Woelders *et al*., 1997), addition of cryoprotectant into cryo-extender (De Leeuw *et al*., 1993).

Commonly, bull sperm for cryopreservation be used the milk extenders, mixtures of skimmed milk and egg yolk, skimmed milk-fructose-egg yolk, whole milk without egg yolk, citric acid-phosphate-egg yolk or Tris based extenders (Barbas and Mascarenhas, 2009). Especially, from among these extender, bull sperm for cryopreservation in domestic industry were widely using the tris basement medium with 20% egg-yolk such as Triladyl® which extender could be applied to other species to ram sperm (Ollero *et al*., 1998), stallion sperm (Blottner *et al*., 2001). The cryoprotectant was important to bull semen cryopreservation in company with cryo-extender. Glycerol is the most widely used cryoprotectant for bull sperm because it reduces the mechanical damage to spermatozoa during the freezing process (De Leeuw *et al*., 1993). Other researchers believe that a low-molecular-weight cryoprotectant, such as ethylene glycol, may cause less damage to sperm than glycerol, because its low molecular weight allows it to cross the plasma membrane more easily (Purdy, 2006). Egg yolk is a strong cryoprotectant agent that has been widely used in the extenders (Hu *et al*., 2010).

DAMAGE BY ROS DURING SEMEN PRESERVATION

ROS are chemically reactive molecules containing oxygen. All organism must metabolize to live, oxygen (O_2) is essential for the survival of all aerobic organisms. Processing of oxidative phosphorylation is produced to aerobic energy in mitochondria. Free radicals generally result from the transfer of one, two or three electron to O_2 to from a superoxide anion ($O_2 \cdot$), hydrogen peroxide (H_2O_2) or a hydroxyl radical ($OH \cdot$) (Aitken, 1995; Alvarez and Storey, 1989; 1993). ROS from metabolism of oxygen is effect to roles in pathway and homeostasis.

It is important to recognize that low levels of ROS play an important to for some physiological reaction, for maturation in male reproductive organ and capacitation in female reproductive organ (Aitken and Vernet, 1997). Sperm can be immature and incapable of fertilization of oocyte when sperm are generated from the testis following spermatogenesis. During capacitation, intracellular pathway molecules mediate lipid redistribution. The aim of this response is to destabilize the plasma membrane and penetrate for oocyte and acrosomal exocytosis the sperm. This processing has been produced that low level ROS was generated by sperm, either through a promoted membrane bound NADPH oxidase from mitochondrial, trigger the signaling for inducement of capacitation (O'Flaherty *et al*., 2006).

Excessive of ROS levels be caused to endogenous antioxidant system that may influenced to effect to harm of male reproductive cell, generation of apoptosis, decreasing of mitochondrial membrane potential, loss of DNA and motility in the sperm (Lopes *et al*., 1998; Sanocka and Kurpisz, 2004). Lipid peroxidation of sperm plasma membrane was formed by ROS attack, processing of lipid peroxidation is breakdown of polyunsaturated fatty acids (PUFAs) that consist a large component of plasma membrane in porcine sperm organelles (Drevet, 2006).

Excessive production of ROS in semen, could be a cause for infertility (de Lamirande and Gagnon, 1995). Excessive concentration of hydrogen damage of DNA, lipid peroxidation protein oxidation and apoptosis result in cell death. ROS and higher levels of caspases-9 and -3, cytochrome c and apoptosis gene (Bax and Bcl) were increased sperm damage, which indicate apoptosis in abnormal animal with 'male factor' of infertility (Wang *et al*., 2003). The H_2O_2 directly influences sperm function unfavourable at fertilization. Low concentrations maintain capacitation such as phosphorylation, acrosome reaction, increasing of calcium ion and activation of inositol 1,4,5-triphosphate whereas high concentration have a harmful effect to, as determined by final purpose of the capacitation process. These effects are likely dependent on modifications of plasma membrane and intracellular homeostasis by the oxidative process (Oehninger *et al*., 1995).

EFFECT OF ANTIOXIDANTS DURING SEMEN STORAGE

The protective antioxidant systems were priority of cell cytoplasmic origin. Sperm were disappeared most of their cytoplasm during the final stage during spermatogenesis, this event caused be insufficiency cytoplasmic component containing antioxidants that counteract the damaging effects of ROS and LPO. Therefore, sperm were delicate cells to LPO during cryopreservation and thawing, leading to subsequent sperm dysfunctions (Alvarez and Storey, 1989; Storey, 1997). Finally, rack of cytoplasmic component was limited antioxidant ability for effect of oxidative stress. The Oxidative stress generally leads to loss of motility, swelling and the blessing of the acrosomal membrane and disruption or increased permeability of the plasma membrane of spermatozoa (White, 1993). Thus, mammalian sperm may be insufficient in preventing oxidative stress such as ROS and LPO for the liquid preservation and frozen-thawing process. To cope with effects of ROS, seminal plasma has antioxidant systems composed of superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH; Aitken and Baker, 2004; Gadea *et al*., 2004).

Mammalian cells can only utilize cysteine which has been shown to easily penetration into the cytoplasmic, high intracellular biosynthesis GSH both *in vitro* and *in vitro* (Mazor *et al*., 1996). L-cysteine is a precursor of GSH which has been shown to improve intracellular GSH production both *in vitro* and *in vitro* (Jeyendran *et al*., 1989) and to prevent hydrogen peroxide-mediated damage of sperm ability in bulls (Bilodeau *et al*., 2000). The L-cysteine has been shown to prevent loss of motility, viability and membrane integrity during sperm liquid storage or in the frozen state (SZCZĘŚNIAK-FABIAŃCZYK *et al*., 2003). In addition, it has improved porcine oocyte maturation and fertilization *in vitro* (Jeong and Yang, 2001). It protect to mechanism against oxidative stress in sperm organelle, protection of membrane and capacitation inhibitor and play a major role as the plasma membrane stabilizers of sperm in many species (Johnson *et al*., 2000).

Under physiological conditions, protection of mammalian sperm against oxidative stress is affected by an enzymatic antioxidant system, mainly represented by SOD and CAT present in sperm and seminal plasma of different species including boar and bull (De Almeida *et al*., 1989; Hermelo *et al*., 1987; Thiangtum *et al*., 2009). CAT catalyzes the decomposition of H_2O_2 into water and oxygen, thus removing an initiator of chain reactions leading to lipid peroxidation and to the formation of other reactive radicals (Aitken, 1995). This enzyme is necessary for disintegration of hydrogen peroxide (H_2O_2) that produced during sperm anaerobic metabolic process (Salisbury and Lodge, 1963). Thus, addition of CAT to semen extenders to overcome the increased production of H_2O_2 has received the attention of such studies dealing with preservation of semen. Addition of the extender with CAT has improved to sperm storage or parameters in several species (Michael *et al*., 2007; Roca *et al*., 2005). Therefore, it may be needed to improve preservation semen using the complex antioxidant system that is the enzymatic antioxidant such as CAT and non-enzymatic and precursor antioxidant such as L-cysteine in domestic livestock industry.

BIOLOGICAL FUNCTION OF MAGNETIZED WATER

Magnetized water is that liquid water is passed though magnet (general magnet or electromagnet). Magnetic fields affect liquid water that hard water by passing it through a magnetic field, as a non-chemical alternative to water softening. Several studies has investigated effect of magnetized water on (Zhou *et al*., 2000), disruption to the hydrogen bonding (Chang and Weng, 2008), weakening the van der Waals bonding between the water molecules (Krems, 2004) and increasing of electronic donor (Tigrek and Barnes, 2010). Especially, magnetic fields have an effect to weakening of van der Waals bonding, between water molecules is formed tightly bond, due to magnetic fields reduce the thermal motion of the inherent charges by dampening forces (Inaba *et al*., 2004). Due to the fine balance between the conflicting hydrogen bonding and non-bonded interaction in water cluster, therefore weakening of the van der Waals attraction leads to a further strengthening of the hydrogen bonding and greater cyclic hydrogen bonded clustering. As physical properties, magnetized water have a special characteristic that easy supercooling (Zhou *et al*., 2012), producing of smaller ice crystals (Woo and Mujumdar, 2010), high electronic donor, increasing of electric conduction and increasing of hexamer structure (Tigrek and Barnes, 2010). Water structures can form vary formation from a sing molecule to clusters of hundreds of molecules bonded together (Tigrek and Barnes, 2010). Water dimers is be simplest structure, after single molecules and molecule clusters is many dimer structure (Keutsch and Saykally, 2001). Especially, hexamer structure is very stable structure of

many others molecule clusters, that theoretical predictions of stabilities of the five lowest energy water hexamer structure (Liu *et al*., 1996).

Magnetized water has been used in many fields and studied by many researchers (Chang and Weng, 2008; Dontas *et al*., 2011; Szkatula *et al*., 2002; Tigrek and Barnes, 2010; Woo and Mujumdar, 2010; Zhou *et al*., 2000). However, application of magnetized water has been mostly used to industrial water in fields. Because of high electronic donor, high hydrogen-bond, increasing electric conduction and smaller formation of ice-crystal, magnetized water have ability on reduction of oxide (SiO_2, Fe_2O_3, CaO, MgO, SO_3, Na_2O, K_2O, CuO, Mn_2O_3, ZnO and CO_2) and reduction of corrosion in pipe (Szkatula *et al*., 2002). In term of industrial water, magnetized water is advantage for industry, however biological application has little studied by magnetized water. From a biological function, application of magnetized water have boundless potentialities for biotechnology. Ability of high electronic donor was suggesting a possible removing of free radical, antioxidant. Formation of stabilizing cluster was suggesting a possible making of smaller water clusters, high absorption and permeability into organism cells. Ability of magnetized water on organism cells may be expected to protection of sperm cell membrane on domestic animals.

CONCLUSION

Betterment of storage technique in domestic animal sperm is resulted high animal production, advancement of breeding system and helping endangered animals that are directly related with improvement of farm household economy and preservation of gene. In domestic animal industry, mostly boar semen was preserved with liquid preservation method and bull semen was cryopreserved into liquid nitrogen under the ultralow temperature condition. In case of boar semen preservation, due to there are short and long term semen diluter, it should select extender with proper purpose. Aspects of bull semen cryopreservation, it is important to regulation of temperature around environment and cooling rate. The oxidative stress was generated during the preservation both boar and bull semen. There are two point to reduce of oxidative stress that supplement of the enzymatic and non-enzymatic complexes antioxidant into diluter or extender and using the magnetized diluter and extender for reducing the free radical during the liquid preservation and minimizing of mechanical damage product smaller ice crystal during the cryopreservation.

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