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The Addition of L-Arginine in Capacitation Media to Motility, Viability, and Spermatozoa Capacity of Goats

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Abstract : The purpose of this study is improving the provision of animal origin food of in vitro fertilization technology. Specific targets to be achieved in this study is to increase the incidence of capacitation spermatozoan and success in in vitro fertilization process . This research utilized four treatment groups, namely controlled group (P0) is the addition of L-Arginine in spermatozoa (HEPES) capacitance medium , group (PI) is the addition of L-Arginine in spermatozoa (HEPES) capacitation medium by 0,002 M / ml ,group (PII) is the addition of L-Arginine in spermatozoa (HEPES) capacitation medium by 0,004 M / ml. and group (PIII) is the addition of L-Arginine in spermatozoa (HEPES) capacitation medium by 0,006 M / ml Further evaluations included motility, viability, capacitation reaction of spermatozoa. Data obtained in the form of percentage of motility, viability and capacitation reaction is analyzed by F Test. The conclusion of this study, group (PIII) is the addition of L-Arginine in spermatozoa (HEPES) capacitation medium by 0,006 M / ml increase of motility, viability and capacitation reaction.
Keywords : L-Arginine, capacitation medium, motility, viability and capacitation reaction.

1.Introduction

Various technologies have been created and have been used to improve the efficiency of livestock reproduction. Artificial Insemination is the initial use of Reproductive Biotechnology in male animals and then followed by research on in vitro manipulation of spermatozoa. How to manipulate spermatozoa in vitro include the method of centrifugation. In the process of in vitro fertilization requires the presence of a mature ovum and spermatozoa who have experienced maturity or capacitation. The maturation process of spermatozoa in invitro can be achieved by adding the appropriate medium[8].

The adverse effects of seminal plasma splitting with centrifugation techniques are the increased formation of Reactive Oxygen Species (ROS) by spermatozoa[11]. The increased accumulation of ROS production in centrifuged spermatozoa is thought to be a complex process and can be derived from various chemical processes, organelles or cells even from outside the cell[2]. If the production of ROS is excessive and

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unable to be neutralized by the antioxidant defense system present in the spermatozoa or seminal plasma can cause damage poly unsaturated fatty acid which is an important component of the phospholipid membrane membrane spermatozoa cells, the inactivation of glycolytic enzymes, motility and spermatozoa[3]. According to[1], L-Arginine is an amino acid that plays an important role in stimulating the motility of spermatozoa in mammals under in vitro conditions. L-Arginine also acts as an antioxidant that produces nitric oxide from enzyme synthesis reactions thus reducing peroxidation of lipid membrane spermatozoa that occurs due to free radicals when spermatozoa interact directly with oxygen. Nitric oxide is a biological molecule that plays an important role in physiological spermatozoa such as spermatozoa motility, spermatozoa-ovum interaction and spermatogenesis. Nitric oxide also plays a role in the defense mechanism of spermatozoa against the formation of reactive oxygen under the freezing point in liquid nitrogen at -196^{o}C, which maintains sperm motility and post thawing viability.

According to[6], L-Arginine is a substrate that produces nitric oxide with an intermediate enzyme nitrate oxide synthase. The enzym is in the acrosome section and the spermatozoa tail. L-Arginine stimulates capacitation and spermatozoa acrosome reaction[11]. At low concentration, NO improve sperm motility. Beside promoting sperm motility, NO is also know to enhance capacitation and acrosom reaction in mouse and human spermatozoa [11;13]. However, higher concentrations of L-Arginin can have adverse effect on motility and fertility of human[14] and rat [23].Research on L-Arginine has been done, but research on the addition of L-Arginine in goat spermatozoa media has not been studied.

2. Materials and Methods

2.1.Experimental animal

Semen is collected from three male goats by using an artificial vagina. The average body weight was 45 kg and average age was 3-4 years. An artificial vagina is prepared by placing both shrouded and sterilized containers, while the space between the outer and inner sheath is filled with warm water at 45 ° C in order to give the temperature to the inner sheath of 42-43 °C and the front third of the envelope in the vaginal artificial Vaseline smeared . After the artificial vagina is prepared, the male is given a stimulus with the angler females then carried out semen storage. Immediately after the shelter, the semen was taken by a laboratory to be separated from the spermatozoa and the plastics were used to be added to the goat semen diluent. The goat semen is accommodated with an artificial vagina and examined macroscopically and microscopically. Macroscopic examination includes volume, color, odor, consistency and pH and microscopic examination including mass movement, individual movement, viability, concentration and test resistance.

2.2. L-Arginine application on spermatozoa capacitation media

Good quality semen (percentages of spermatozoa motility and viability should be 70% or above), as much as 0.5 ml plus HEPES medium 1 ml and centrifugation at 1800 rpm for 10 minutes. Calculated 3x10^{6} spermatozoa for treatment.Spermatozoa of each centrifugation divided by 4 groups.Tube I filled with spermatozoa and HEPES medium, tube II filled with spermatozoa + HEPES medium + L-Arginin 0.002 M / ml HEPES. Tubes III filled with spermatozoa + HEPES medium + L-Arginin 0.004 M / ml HEPES. Tubes IV filled with spermatozoa + HEPES medium + L-Arginin 0.006 M / ml HEPES.Then incubated for 15 minutes at room temperature and evaluated for motility, viability and capacitation.

2.3. Assesment of motility

The motility of spermatozoa was analyzed by mixing the semen gently and placing a 10µl drop of diluted semen on warm slide covered with a glass cover slip (18 x 18 mm) from five selected representative fields. One hundred spermatozoa were evaluated in at least five different fields in each smear under a light microscope. Individual sperm were recorded as being motil or non motil [21].

2.4. Sperm viability

Eosin – negrosin staining was used to evaluate sperm viability as described by[21]. One drops of semen was placed on a tempered glass slide and this samples was mixex with one drop of eosin negrosin solution. The mixture was smeared on the glass slide and allowed to air dry. One hundred spermatozoa were evaluated in at

least five different fields in each smear under a light microscope. Eosin penetrates non viable, non stained cells [21].

2.5. Assesment of sperm capacitation with Tetracycline Chlorine Staining

Examination of sperm capacitation with ChlorTetracyclin (CTC) staining. Preparations are examined under a fluorescent microscope with a magnification of 400x. The visible picture is: the whole head of the spermatozoa is fluorescent, the spermatozoa are non-capacitated. The upper half spermatozoa head fluorescent is a spermatozoa that undergoes capacitation.

2.6. Design and Statistical Analysis

The research design used is complete randomized design and data analisis done with Anova Test, if there is difference followed by Duncan Test [17].

3. Result

3.1.Collection of Goat semen for Treatment

This research requires fresh semen of goats that are first examined in macroscopic and microscopic. The macroscopic examination includes: volume, color, odor, consistency and degree of acidity or pH. Microscopic examination includes mass movement, individual movement, concentration and survival (viability) of spermatozoa. The results of the macroscopic and microscopic examination of goat's semen (Table 1).

Table 1. Macroscopic and microscopic examination of fresh goat semen

Indicator	Character
Colour	White-yellowish
Smell	Typical
Consistency	Thick
pH	7,00
Volume (ml)	2,5±0,35
Conceentration (juta) spz/mm^3	3985x10^6
Mass Motility	+++
Individual Motility (%)	Progressive (87±3,50)
Viability (%)	93±2,45
Whole Plasma Membrane (%)	85±1,55

The results showed that the goat semen that was accommodated was yellowish white, typical smell, thick consistency, pH ± 7, volume 2.5 ± 0.35 ml, concentration 3985 ± 106, mass motility +++ (movement of large and large waves) , individual motility progressive move 87 ± 3.50%, viability 93 ± 2.45 and intact plasma membrane 85 ± 1.55%. In general, the volume of semen will increase according to age, body size, changes in state, reproductive health and frequency of semen shelter. The color, consistency and concentration of spermatozoa are closely related to each other. The more dilute a semen the concentration of spermatozoa will be lower and the color of the semen is getting pale. While the consistency of semen depends on the ratio of spermatozoa and plasma semen [4]. The degree of acidity (pH) greatly affects the life-force of spermatozoa. When a high or low pH will cause the spermatozoa die. The degree of acidity of semen is influenced by the concentration of lactic acid produced in the metabolic process. According[8], spermatozoa metabolism in anaerobic state will produce lactic acid buried and increase or decrease the pH of semen.

3.2 Examination of motility, viability and capacitation of goat sperm in HEPES + L-Arginine media

Individual motility was observed using a 400 times magnification light microscope. Observations were made on the number of progressive motile spermatozoa and spermatozoa velocity from one field of view and expressed in percent can be seen in table 2.

Observation of spermatozoa viability was done using a light microscope with 400 times magnification. The percentage of viability of spermatozoa can be seen in table 2. The live spermatozoa are characterized by clear or transparent colored spermatozoa heads while the dead spermatozoa are characterized by pink spermatozoa heads.

The observed capacities and acrosome reactions were performed by Chlortetracyclin (CTC) staining and were observed with a magnification fluorescence microscope 400 times. The visible picture is: the whole head of the spermatozoa is fluorescent, the spermatozoa are not capacitated. The upper half-fluorescent spermatozoa head is a spermatozoa that has matured or capacitated. Observations were made from one field of view and expressed in percentages can be seen in table 2.

Table 2.Percentage of motility, viability and capacitation of goat spermatozoa in HEPES-added L-Arginine media.

Parameter	**HEPES**	**HEPES + 0,002 M/mL L-Arginin**	**HEPES + 0,004 M/mL L-Arginin**	**HEPES + 0,006 M/mL L-Arginin**
Motility P (%)	50,10±2,55[a]	35,05±2,25[b]	56,75±1,50[a]	68,35±3,05[c]
Viability (%)	58,35±2,30[a]	44,55±1,05[b]	65,75±2,50[a]	72,25±1,75[c]
Capacitation (%)	54,45±0,75[a]	37,45±2,25[b]	60,45±0,80[a]	82,55±0,85[c]

Different letter notations on the same line are significantly different ($p < 0.05$)

In Table 2 it can be seen that the addition of L-Arginine in the capacitation medium (HEPES) yields a distinct pattern of difference in progressive motility, viability and spermatozoa capacitance. Motility, viability and spermatozoa capacitation in HEPES + medium 0.002 M / mL L-Arginine showed the lowest percentage. Between medium HEPES and HEPES + 0,004 M / ml L-Arginine there was no difference, meanwhile the highest on medium HEPES + 0,006 M / ml L-Arginin.UjiMultivariant to motility, viability and spermatozoa capacitation there was significant difference $p<0,05$ between the treatments.

4. Discussion

The movement of spermatozoa can occur because of the energy (ATP) that is product by mitochondria and the existence motor dynein (Cytoskeleton) for flagela or tail of sperm. The movement organized by Ca^{2+} (Cyclic Adenosin Mono Phosphat)[22]. Absolute spermatozoa survival require the integrity of the membrane. During the centrifugation process in addition to the antioxidant present in the seminal plasma disappeare there is also membrane molecular materials resulting in membrane destabilization thereby decreacing the physiological function of the membrane under normal condition, living cells need a source of energy formed from ATP through oxidative fosforylation requiring oxygen in process of reduction of oxygen in the transfer 4 electron. Inextain situation this transfer of 4 electron is running less than perfect, so that the reactive oxygen compound is very dangerous and damage the cells if not muted. The reaction oxygen compound is power full oxidant than can damage cell components that are essential to maintain cell integrity in live [20].

Capacitation showed a correlation with change in fluidity of plasma membrane spermatozoa, intracellular ion concentration, metabolism and motility[22]. Molecularly during capacitation there are modifications of calcium intracellular ions and other ions, changes in lipid and phospholipid membrane spermatozoa, changes in protein phosphorylaion and activity of protein kinase. Capacitation is the re-arrangement of biochemical membrane spermatozoa where as capacitance increases in intracellular Ca^{2+} level [15]. The increasing of Ca^{2+}activity activates spermatozoa (hyperactivation).

The results showed that the highest percentage of motility, viability and capacitance of spermatozoa in HEPES + 0,006M / ml L-Arginine. The statement is appropriate with the research [6], which writing L-Arginine can acts as an antioxidant that protects spermatozoa from free radicals and lipid peroxidation so that the spermatozoa plasma membrane remains intact. In addition, because the dose of L-Arginine given in the medium of capacitance is to balance the occurrence of lipid peroxidation or free radicals.

L-Arginine is an amino acid that can stimulate the motility of mammalian spermatozoa under in vitro conditions and play an important role in the defense of cellular immunity [19]. L -Arginin protects spermatozoa against lipid peroxidation by increasing the production of nitric oxide and deactivating free radicals. Nitric

oxide has been shown to be an antioxidant and beneficial to reactive oxygen species (ROS) such as hydrogen peroxide (H202) and superoxide anion (O2) [1].The mechanism of L-Arginin action is through the production of NO in the presence of NOS, and it was confirmed by the inhibition of the enzyme action as well as by scavenging the NO free radical. A similar role of NO has been demonstrated in human spermatozoa where this ROS has been shown to trigger tyrosine phosphorylation and double serine/threonine phosphorylation (Herrero et al, 2000) both events being closely realted with capacitation[10]. Moreover, [9]and [7]mentioned that the arginin acts as an antioxidant which protects spermatozoa against lipid peroxidation during storage through increasing nitric oxide production which reduces lipid peroxidation by activating free radicals. L-Arginine is a secondary antioxidant that works by chopping off a chain-oxidation reaction from free radicals or by capturing free radicals. L-Arginine can also increase spermatooza glycolysis. The process of glycolysis can produce energy in the form of adenosine triphosphate (ATP), which is used by spermatozoa as a source of energy in the process of movement so that it can remain motile and maintain its life force.Futhermore, [5]reported that the nitric oxide have a major role in stimulation of lactic dehydrogenize which was a key for metabolic process in sperm motility that may be presumably given as the sperm direct effects responses individual motility.

The conclusion of this study, group (PIII) is the addition of L-Arginine in spermatozoa (HEPES) capacitation medium by 0,006 M / ml increase of motility, viability and capacitation reaction.

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