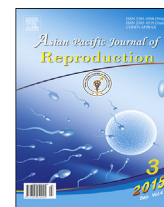
Contents lists available at ScienceDirect

Asian Pacific Journal of Reproduction

journal homepage: www.apjr.net



Original research http://dx.doi.org/10.1016/j.apjr.2015.06.003


# The effects of insulin-like growth factor I (IGF-I) complex from seminal plasma on capacitation, membrane integrity and DNA fragmentation in goat spermatozoa

Suherni Susilowati[1], Indah Norma Triana[1], Abdul Malik[2*]

[1]Department of Veterinary Reproduction, Faculty of Veterinary Medicine, Airlangga University, Surabaya, Indonesia

[2]Department of Animal Science, Islamic University of Kalimantan, Banjarmasin, Indonesia

ARTICLE INFO

*Article history:*
Received 2 Mar 2015
Received in revised form 21 May 2015
Accepted 26 May 2015
Available online 24 July 2015

*Keywords:*
Spermatozoa
IGF-I complex
Seminal plasma
Membrane integrity
DNA fragmentation

ABSTRACT

To evaluate the effects of the insulin-like growth factor I (IGF-I) complex from seminal plasma on capacitation, membrane integrity and DNA fragmentation.

**Methods:** A total of 0.5 mL of fresh semen was added to 1 mL of Bracket–Oliphant (BO) medium, and the sample was then centrifuged at a speed of 1 800 rpm for 10 min. The samples were analyzed before and after centrifugation for sperm viability, motility, membrane integrity and capacitation. The centrifuged samples were divided into three groups, each consisting of $3 \times 10^6$ spermatozoa. BO medium was added to group 1, BO + 12 ng IGF-I complex medium was added to group 2, and 12 ng IGF-I complex was added to group 3. Then, the samples were incubated for 15 min.

**Results:** The result showed that sperm motility, viability and membrane integrity were significantly higher ($P < 0.05$) after centrifugation. Furthermore, the sperm capacitation was significantly lower ($P < 0.05$) after centrifugation. The percentages of sperm capacitation, membrane integrity and DNA fragmentation were significantly different ($P < 0.05$) in all media, including BO, BO + IGF-I complex and the IGF-I complex alone.

**Conclusions:** Sperm quality include motility, viability and membrane integrity were lower after centrifugation. Whereas DNA fragmentation after incubation in the IGF-I complex medium also was lower compared to that of specimens in the BO and BO + IGF-I complex media.

## 1. Introduction

Sperm manipulation for improving fertilization is undertaken not only *in vivo* but also *in vitro* and underlies the manipulation of spermatozoa for clinical *in vitro* fertilization (IVF). One method for sperm manipulation is the centrifugation of spermatozoa. The process of capacitation of spermatozoa involving biochemical and physiological processes involves complex reactions. During capacitation, modification and characterization of the membrane occur, in addition to enzyme activation and spermatozoa motility [1]. One negative result of centrifugation of semen is the increased formation of reactive oxygen species (ROS) by the spermatozoa. The increase in ROS production after separation by centrifugation is thought to be a complex process and can be derived from chemical processes in organelles inside the cell or even from processes outside the cell [2]. Reactive oxygen species are an important mediator of the function of spermatozoa and are involved in hyperactivity induction, capacitation and acrosome reaction, in addition to spermatozoa and oocyte fusion [3]. However, when excessive ROS are produced, they cannot be neutralized by the antioxidant defense systems of spermatozoa or seminal plasma. This excess will lead to fatty acid damage, especially among polyunsaturated fatty acids, which are essential components of the sperm membrane phospholipid layer, the inactivation of glycolytic enzymes, DNA chain termination, and a decrease in sperm motility and sperm death.

Semen consists of spermatozoa suspended in a fluid medium called seminal plasma. Seminal plasma is a complex fluid that mediates the chemical function of the ejaculate. One component of seminal plasma is insulin-like growth factor (IGF-I). This growth factor has been suggested to have a direct or indirect role in spermatogenesis/steroidogenesis in the testes, and its derangement may be involved in male infertility [4,5]. The protein contained in seminal plasma includes the insulin-like growth factor I (IGF-I) complex.

*Corresponding author: Abdul Malik, Department of Animal Science, Islamic University of Kalimantan, Banjarmasin, Indonesia
E-mail: sidol_99@yahoo.com
Peer review under responsibility of Hainan Medical College.

2305-0500/Copyright © 2015 Hainan Medical College. Production and hosting by Elsevier (Singapore) Pte Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Insulin-like growth factor-I forms a complex that binds with another molecule that has a molecular weight of 150 kDa and consists of three protein molecules, including one molecule of insulin-like growth factor-I (sub unit α) with a molecular weight of 7.6 kDa, one molecule of insulin-like growth factor binding protein (sub unit β) with a molecular weight of 53 kDa and one molecule of an acid-labile subunit with a molecular weight of 85 kDa (sub unit β) [6]. The acid-labile subunit that binds with IGF-I increases the molecular weight by adding a complex function to the bond between IGF and IGF-3. In seminal plasma, IGF-I has been identified in the testes and is secreted by Leydig and Sertoli cells [7]. IGF-I has been reported to be a significant factor for germ cell development, maturation and the motility of spermatozoa [8,9].

Selvaraju *et al.* [10] reported that variations in IGF-I levels in the seminal plasma can influence germ cell stimuli, including the development, maturation and motility of the spermatozoa. Hence, this study was conducted to determine the possibilities of improving sperm quality with the insulin-like growth factor I (IGF-I) complex after incubation. The objective of the present study was to evaluate the effects of the insulin-like growth factor I (IGF-I) complex from seminal plasma on capacitation, membrane integrity and DNA fragmentation in goat spermatozoa.

## 2. Materials and methods

### 2.1. Experimental animals

A total of three male goats were used for semen collection in this study. The average body weight was 45 kg, and the average age was 3–4 years. Semen was collected from the goats two weeks after start of adaptation to the location with the aid of an artificial vagina. Immediately, after collection, the semen was kept in a water bath (37 °C), and semen parameters were assessed, including volume, pH, consistency, color and concentration of the semen. A total of 0.5 mL of fresh semen was added to 1 mL of Bracket–Oliphant (BO) medium, and the sample was then centrifuged at a speed of 1 800 rpm for 10 min. The samples were analyzed before and after centrifugation for sperm viability, motility, membrane integrity and capacitation. The centrifuged samples were divided into the three groups, each consisting of $3 \times 10^6$ spermatozoa. BO medium was added to group 1, BO + 12 ng IGF-I complex medium was added to group 2, and 12 ng IGF-I complex was added to group 3. Then, the samples were incubated for 15 min. The samples were then analyzed for sperm viability, motility, membrane integrity, capacitation and DNA fragmentation. For identification, constituents from goat seminal plasma proteins were assessed via native polyacrylamide gel electrophoresis (Native-PAGE) with a concentration of 12% using an electrophoresis mini protein gel (Bio-Rad), and the IGF-I complex protein was isolated from seminal plasma by electro-elution.

### 2.2. Sperm viability

Eosin-nigrosin staining was used to evaluate sperm viability as described by Malik *et al.* [11]. After thawing, one drop of semen was placed on a tempered glass slide, and this sample was mixed with one drop of eosin-nigrosin solution (0.2 g of eosin and 2 g of nigrosin were dissolved in a buffered saline solution, mixed for 2 h at room temperature and filtered to obtain the staining media). 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 cells, which appear red, and nigrosin offers a dark background for facilitating the detection of viable, non-stained cells.

### 2.3. Assessment of motility

The motility of the spermatozoa was analyzed by mixing the semen gently and placing a 10 μL drop of diluted semen on a warm slide covered with a glass cover slip (18 × 18 mm) from five selected representative fields. Samples were selected randomly from 10 fields, for a total of 200 cells. Individual sperm were recorded as being viable or dead.

### 2.4. Assessment of sperm membrane integrity

Membrane integrity was determined using the hypo-osmotic swelling test (HOST) described by Malik *et al.* [12]. A total of 100 μL of semen was mixed with 1 mL of hypotonic solution (osmotic pressure 100 mOsm/kg) containing 13.51 g of fructose and 7.35 g of sodium citrate in 1 000 mL of distilled water. The mixture was incubated at 37 °C for 60 min. Following incubation, 15 μL of the sample was placed on a slide, covered with a cover slip and observed under a differential interference microscope (Olympus CK2, ULWCD 0.30) at a magnification of 400×. The spermatozoa were categorized according to the presence or absence of a swollen tail. At least 200 spermatozoa were observed, and the results were recorded as percentages. The membrane integrity after HOST was classified into two groups: normal spermatozoa that displayed coiled tails and abnormal spermatozoa without coiled tails.

### 2.5. Assessment of sperm capacitation

Sperm capacitation was assessed using chlortetracycline (CTC). Coloration with CTC showed that when the spermatozoa underwent visible capacitation, 2/3 of the equator appear to be yellow sperm heads that are lighter due to the increased distribution of $Ca^+$, the spermatozoa that underwent acrosome reactions were colorless and had only the yellow tape on the equator of the spermatozoa head. Observations of the capacitation and acrosome reaction of the spermatozoa were performed using a fluorescence microscope at a magnification of 400×.

### 2.6. Assessment of sperm DNA fragmentation

DNA fragmentation was assessed by a terminal deoxynucleotide transferase-mediated dUTP nick end labeling (TUNEL) assay adopted by Natalia-Rougier *et al.* [13]. Briefly, all elements were fixed in 2% formaldehyde in 1× PBS solution (pH 7.4; Gibco) for at least one hour. Each sample was placed into one well of a multiwell plate (4-mm diameter). After 2–3 h, each well was washed with 1× PBS (three times, 5 min each), and the cells were permeabilized with cold methanol. Before incubation with the TUNEL solution, each well was washed again with 1× PBS. For each sample, one extra well was incubated with DNA (1 U/mL; Sigma) for 30 min at 37 °C as a positive control, and in another well, the TUNEL

Download English Version:

https://daneshyari.com/en/article/3453608

Download Persian Version:

https://daneshyari.com/article/3453608

Daneshyari.com