



# THE HYPO-OSMOTIC SWELLING TEST IN FRESH GARUT RAM SPERMATOZOA

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## ABSTRAK

Penelitian ini bertujuan untuk menentukan waktu optimum untuk pengujian membran plasma yang utuh (MPU) pada sperma domba garut setelah inkubasi pada larutan hypo-osmotic. Sebanyak 24 ejakulat dari 8 ekor semen domba garut yang telah dewasa kelamin dikoleksi menggunakan vagina buatan. Semen dievaluasi secara makroskopis selanjutnya dilakukan pengujian MPU dengan mengambil 10 µL semen segar dan dimasukkan ke dalam 2 mL larutan hypo-osmotic kemudian diinkubasi pada suhu 37°C selama 1 jam. Sample dievaluasi setiap 15 menit dengan menghitung 200 sperma yang bereaksi (coil) dan yang tidak bereaksi dari lima lapang pandang. Hasil penelitian menunjukkan waktu optimal pengujian MPU menggunakan larutan hypo-osmotic adalah 30 menit dan tidak ada perbedaan antar 8 domba jantan yang digunakan

*Kata Kunci : Keutuhan membran plasma, Semen domba garut, HOS test*

## ABSTRACT

The study was aimed to determine the optimal time to evaluate the maximum numbers of swollen sperm after expose to hypo-osmotic solution for testing membrane integrity in fresh garut ram semen. A total of 24 ejaculate samples from eight sexually mature garut ram were collected using artificial vagina. After macroscopic evaluation, 10 µL undiluted semen was gently mixed in each of the 2 mL hypo-osmotic solution and incubated at 37°C for 1 hour. Two hundred of coil and non-coiled sperm were observed from five different fields every 15 minutes. Results demonstrated that the maximum percentage of coiling sperm positive to HOS test was at 30 minutes and no differences among 8 garut rams used.

*Keywords: hypo-osmotic swelling test, garut rams semen, membrane integrity*

## INTRODUCTION

The plasma membrane surrounds the entire sperm cell holding together its organelles and intracellular components. Viable sperm are defined as cells that possess an intact plasma membrane. Membrane integrity is a fundamental requisite for sperm viability and for the success of fertilization. In recent years more attention has been dedicated to study this area. Two tests have been available to evaluate membrane integrity supravital stains and the hypo-osmotic swelling (HOS) test.

The hypo-osmotic swelling test has proved to be a good tool for evaluating the membrane

integrity of sperm of various domestic animals including bull (Revell and Mrode, 1994; Rota *et al.*, 2000; Brito *et al.*, 2003; Bacinoglu *et al.*, 2008; Padrik *et al.*, 2012), stallion (Neild *et al.*, 1999; Nie and Wenzel, 2001; Eshleman and Pinto, 2010), and boar (Vazquez *et al.*, 1997; Perez-Llano *et al.*, 2001, 2003; Yeste *et al.*, 2010), dog (Dobranić *et al.*, 2005; Tomislav *et al.*, 2005), buck (Fonseca *et al.*, 2005; Leboeuf *et al.*, 2006), deer (Nalley, 2011) and rabbit (Amorim *et al.*, 2009). Each species of animal contains a different membrane composition (Miller *et al.*, 2005), therefore the characteristics of membranes that affect their sensitivity include the response to hypo-osmotic solution is different.

When sperm encounter hypo tonic environments, they react to hypoosmolality by developing bent or curled of sperm tails due to the influx of water during reestablishment of osmotic equilibrium. In the classic HOS test this property is used to characterize membrane integrity (Jeyendran *et al.*, 1984). However, a wide range of animal cell types, including sperm, are able to maintain their volume after osmotic shock, thereby avoiding the consequences of excessive volume changes (Lang *et al.*, 1998).

Since there were differences among sperm membrane composition, the percentage of sperm cells response to HOS test varies highly in its degree. The present study aimed to determine the optimal time to evaluate maximum numbers of swollen/coiling sperm of fresh garut ram semen after expose to hypo-osmotic solution in for membrane integrity testing.

## MATERIALS AND METHODS

### Semen Collection and Evaluation.

Twenty four ejaculates (8 ram, 3 sample per ram) were collected using an artificial vagina, in the presence of oestrous ewe. Semen samples were placed in a water bath at 32°C. Immediately after the collection, semen volume, pH, color, consistency, mass activity, sperm motility, viability, sperm concentration, and percentage of sperm with morphologic abnormalities were evaluated according to standard procedures (Arifiantini, 2012).

### Hypoosmotic swelling test

The hypoosmotic solution (150 mOsm/L) was prepared by dissolving 7.35 g sodium citrate ( $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$ ) and 13.51 g fructose 7.35g in 1000 mL of distilled water (Jeyendran *et al.*, 1984). The solution was stored at 4°C till used. A volume of 10  $\mu\text{L}$  undiluted semen was gently mixed in each of the 2 mL hypo-osmotic solution and incubated at 37°C for 1 hour. Numbers of swollen sperm was observed every 15 minutes; a drop of incubated suspension was placed on a glass slide, covered with coverslip and examined at under a microscope at 400 x magnification. At least 200 sperm cells on each slide were counted randomly.

### Statistical Analysis

Data presented as the mean and standard deviations (SD) were analyzed by a one way

analysis of variance (ANOVA). Statistical significance was set at  $P < 0.05$  (Santoso, 2003 )

## RESULTS AND DISCUSSION

The characteristics of the ejaculates collected for analyses are reported in Table 1 mean values recorded for semen quality parameters were in agreement with standard reproductive performance of ram.

At the beginning of the incubation period (0 minutes) sperm not affected by hypo-osmotic solution, coiling seen only  $15.29 \pm 0.88\%$ . The mean percentages of sperm with intact membrane determined by the HOST 15 min, HOST 30 min, HOST 45 min and 60 min were  $38.16 \pm 3.89$ ,  $69.47 \pm 2.78$ ,  $42.85 \pm 4.30$  and  $29.95 \pm 3.80$  respectively. Ram sperm appeared to suffer increasing coiling from 15 to 30 minutes, and reached maximum values at 30 minutes and coiling began to decrease after 45 minute (Table 2).

The sperm plasma membrane is susceptible to damage caused, for example, by osmotic stress or lipid peroxidation. The physical pressure from osmotic stress results in membrane damage, but, if the limits to membrane integrity were not exceeded, the plasma membrane will respond, behaving as an ideal osmometer. The hypo-osmotic Swelling test is based on that principle. Thus, when samples are places in a hipoosmotic solution sperm with intact and functional membranes will swell and present typical coiled tails.

Table 1. Characteristic of Garut Ram Semen

Semen Variable	Mean $\pm$ SD
Volume (mL)	0.65 $\pm$ 0.32
pH	6.31 $\pm$ 0.29
Color	milky to cream
Consistency	moderate
Mass activity	2.91 $\pm$ 0.20
Sperm motility (%)	72.92 $\pm$ 4.08
Sperm viability (%)	84.14 $\pm$ 5.28
Sperm concentration ( $\times 10^6$ / mL)	3052.08 $\pm$ 692.11
Total sperm number ( $\times 10^6$ )	1998.86 $\pm$ 1204.27
Sperm abnormality (%)	6.18 $\pm$ 2.04

Table 2. Percentages of Swollen Sperm (8 Ram) Subjected to HOST during One Hour Incubation Period at 37°C

	Time (minute)				
	0	15	30	45	60
% Swollen Sperm	15.29 ± 0.88 <sup>e</sup>	38.16 ± 3.89 <sup>c</sup>	69.47 ± 2.78 <sup>a</sup>	42.85 ± 4.30 <sup>b</sup>	29.95 ± 3.80 <sup>d</sup>

Different superscript at the same raw indicate significant differences (p <0.05)

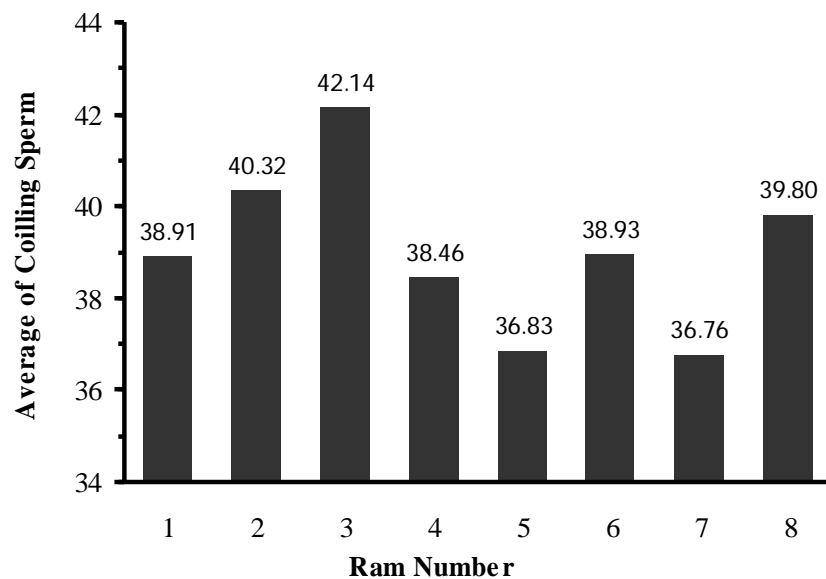


Figure 1. Average Percentage of Total Coiling in Fresh Ram Sperm (200 Cells Counted per Sample) Exposed to Hypoosmotic Solution for 60 Minutes

As with other domestic species, garut ram sperm had a similar pattern of swelling when exposed to a hypoosmotic medium. An important property of the sperm cell membrane is its ability to permit selective transport of molecules. When exposed to hypoosmotic conditions, water will enter the sperm cell in an attempt to reach osmotic equilibrium.

In reacting to the sperm, HOS test is an influx of water into the interior of cells and consequent alteration of cell volume and plasma membrane and causes a folding of the flagellum. Sperm with intact membranes absorb water and swell by increasing in volume to establish equilibrium between the fluid compartment within the sperm and the extracellular medium due the biochemically-active sperm. The hypo-osmotic swelling test is inexpensive and easy to perform,

and evaluate a fundamental characteristic of the plasma membrane.

In this research no difference was observed between rams after HOS test was carried out for 60 minutes at 37°C (Figure 1), and the best time to evaluate HOS test for fresh semen of ram sperm was at 30 minutes incubation this finding was agreement with Vazquez *et al.* (1997) in boar semen 30 minutes incubation was the best compare to 5, 60 or 120 minutes. The incubation time to evaluate membrane integrity with hypoosmotic solution is deferred among researchers. Amorim *et al.* (2009), Nur *et al.* (2005), Lodhi *et al.* (2008), Eshleman and Pinto (2010) and Padrik *et al.* (2012) proposed 1 hour incubated in rabbit, goat, buffalo, equine and bovine semen. Leboeuf *et al.* (2006) and Rota *et al.* (2000) only 5 minutes in goat and bovine semen. Nie and Wenzel (2001)

reported no differences between 1 or 60 minutes in Contreras Eshleman and Pinto (2010) reported 1 minutes was better than 60 minutes in stallion semen.

The incubation time seem to be not the only factor influencing the number of coiling sperm. The osmolarities of hypo-osmotic solution affect the number of coiling sperm tail. The best osmolarities solutions was 60 mOsm/L was found for rabbit sperm (Amorim *et al.*, 2009), 125 mOsm/L for goats semen (Fonseca *et al.*, 2005), 100 mOsm/L for dog sperm (Tomislav *et al.*, 2005), 25 to 100 mOsm/L for horse sperm (Neild *et al.*, 1999) and 150 mOsm/L for cattle, human and swine's (Revell and Mrode, 1994; Jeyendran *et al.*, 1984; Vazquez *et al.*, 1997). According to Petrunkina *et al.* (2007), sperm cell able to maintain their cellular functionality in the face of such osmotic changes, sperm of several mammalian species (boar, mouse, bull, human) have been found to exhibit volume regulatory abilities, particularly regulatory volume decrease (RVD) in response to hypotonic challenge and after exposure to hypertonic conditions, the cells are able to recover their volume after initial shrinking, a process known as regulatory volume increase.

In this research the maximum coiled sperm ( $69.47 \pm 2.78\%$ ) on 150 mOsm/L demonstrated at 30 minutes after incubation, and decreased after 45 ( $42.85 \pm 4.30\%$ ) and 60 minutes ( $29.95 \pm 3.80\%$ ). This phenomenon explains that the RVD of ram sperm demonstrated at that time. Since the osmolarities of hypo-osmotic solution affect the number of coiling sperm tail, this justifies the need for finding the best time to evaluate maximum response of sperm for membrane integrity when using different solution.

## CONCLUSION

The best time to evaluate the garut ram sperm membrane integrity was 30 minutes after incubation.

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