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The Detailed Motility and Velocity Characteristics of Rams Spermatozoa as Assessed by Computer-Aided Semen Analysis

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ABSTRACT: The aim of the present study was to determine the effect of incubation time in TALP medium on the detailed motility and velocity of spermatozoa characteristics. Four rams (R9, R12, R13, R16) of proven fertility were used in this study. Semen was collected by electroejaculation. Rams semen was collected with 4 replicates, respectively to analyzed the motility and velocity characteristics by using the computer-aided semen analysis (CASA). Data were analyzed using SPSS software program. The results showed that percentage of motile, progressive and rapid were significantly decreased from 0 to 120 minutes incubation. In contrast, percentage of static was significantly increased from 21.6% to 71.3% at 0 to 120 minutes incubation. Ram 12 had the best motile with 76.3% at 0 minute incubation and 40.3% at 120 minutes incubation. Average path velocity (VAP), progressive velocity (VSL) and track speed (VCL) at 0 incubation were 91.2 μm/s, 72.2 μm/s and 134 μm/s afterward decreased to 49 μm/s, 40.8 um/s and 77.7 μm/s at 120 minutes incubation, respectively. In conclusion, rams and incubation time were influenced the motility and velocity of spermatozoa.

Keywords: Motility, Velocity, Rams Spermatozoa.

INTRODUCTION

Motility and longevity of sperm are an important factor for sperm to reach the oviducts for fertilization. In a recent study, subjective motility of sperm was investigated in a number of species. Progressive motility appears essential for the passage between the processes and folds of the utero-tubal junction before ovulation. Recent studies by using computer-aided semen analysis (CASA) to define motility characteristics in a number of species including goats (Batista *et al*., 2002), sheep (Bag *et al*., 2002), and camels (Al-Qarawi *et al*., 2002)

Traditionally, motility of spermatozoa has been assessed by visual estimation using a microscope. The element of subjectivity and its attendant drawbacks of human error and bias, is introduced when using this approach. Realizing this disadvantage, attention has been given in recent years to objective methods of evaluating sperm motility by using photomicrography, videomicrography, and automatic motility analyzer The aim of the present study was to determine the effects of incubation time in TALP medium on the detailed motility and velocity sperrmatozoa characteristics, analyzed using CASA.

MATERIAL AND METHODS

Animal

Four rams (R9, R12, R13, R16) of proven fertility were used in this study. Semen was collected by electroejaculation using standard procedures (Ismaya, 2014). Rams semen was collected with 4 replicates, to analyzed the detailed motility and longevity.

Media

In this study, modifications of sperm-TALP (Tyrode's-albumin-lactate-pyruvate) medium was used for sperm culture (Ismaya, 2003) consisted of 100 mM NaCl, 3.1 mM KCl, 2 mM $CaCl_2$, 0.4 mM $MgCl_2$, 25 mM $NaHCO_3$, 21.6 mM L-Lactic acid, 10 mM HEPES, 1 mM Sodium pyruvate, and bovine serum albumin (BSA fraction-5) 6 mg/ml was prepared to dilution of semen at 39^{o}C.

Semen handling and semen dilution

Semen was collected into 15 ml sterile a plastic centrifuge tube (Rohre/tube, Sarstedt, Germany) and a placed into polystrylene box warmed to 39°C by bottles of warm water. The study was conducted on semen from four rams and was repeated three times. The interval time between collection of semen and first analysis of spermatozoa in CASA was about 5 minutes.

Motility, velocity and sperm head analysis

Fifty micro litter of fresh semen from each semen sample was diluted in small tube to 600 ul in TALP medium. The study was conducted on semen from four rams and repeated three times. Sperm motility, velocity and morphology characteristics analysis was performed using computer-aided semen analysis (CASA) system (Hamilton Thorne Research version 10, Beverly MA, USA). Briefly, a 2 µl aliquot of sample was placed in the micro cell chamber, 20 micron (La Jolla CA, USA). At least 200 spermatozoa were counted with CASA to evaluate the sperm motility and velocity. Sperm motility and velocity variables including: percentage of motile, progressive, rapid sperm and average path velocity (VAP), straight-line velocity (VSL).

Statistical analysis

Data were analyzed using SPSS software program (SPSS 11.0 Brief Guide, New Jersey). Data effect of incubation time on motility and velocity were analyzed using analysis of variance one way classification, and the level of significance was considered $P \leq 0.05$. The differences between means was tested by least significant difference test (LSD).

RESULTS AND DISCUSSION

Detailed motility and velocity of ram spermatozoa in TALP medium

Detailed percentage of motile, progressive and rapid at 0 incubation were 78.4%, 54.5% and 64.1% (Figure 1), whereas at 120 minutes incubation the percentage of motile, progressive and rapid were 29%,12.5% and 15.4%, respectively. The results showed that percentage of motile, progressive and rapid were significantly decreased from 0 to 120 minutes incubation. In contrast, percentage of static was significantly increased from 21.6% to 71.3% at 0 to 120 minutes incubation. Percentage of motile and rapid at 0 and 30 minutes incubation were not significantly different, contrary percentage of progressive at 0 and 30 minutes incubation were statistically different ($P \leq 0.05$). Incubation time was influenced the rapid of sperm, average rapid of sperm decreased from 64.1% to 15.4% at 0 to 120 minutes incubation.

At 120 minutes incubation VCL rate of 0 to 20 µm/s was highest contrary with VCL rate at more than 180 µm/s the highest percentage of VCL was at 0 incubation. The VCL of sperm varied between 0 to 180 µm/s and influenced by incubation time.

Ram 13 had VAP of 101 µm/s, ram 9 (95.1µm/s), ram 12 (88 µm/s) and ram 16 (80.6 µm/s) at 0 minute incubation afterward decreased to 45.4 µm/s, 42 µm/s, 57.4 µm/s and 51 µm/s at 120 minutes incubation, respectively. At 0 minute incubation ram 13 and ram 9 had VSL of 92.9 µm/s and 80.5 µm/s respectively, whereas ram 12 and ram16 had lower, there was 60.9 µm/s and 55.1 µm/s, respectively. At 0 minute of incubation ram 9, 12, 13 and 16 had VCL of 149 µm/s, 142 µm/s, 136 µm/s and 109 µm/s respectively afterward decreased to 65.4 µm/s, 87.4 µm/s, 74.1 µm/s and 84 µm/s at 120 minutes of incubation, respectively.

Some factors that influenced of sperm motility in vitro were quality of semen, media of semen, pH of medium, temperature, and morphology of sperm (Ismaya, 2014). In this study showed that rams and incubation time influenced the motility and longevity of spermatozoa in diluted semen at 39°C.

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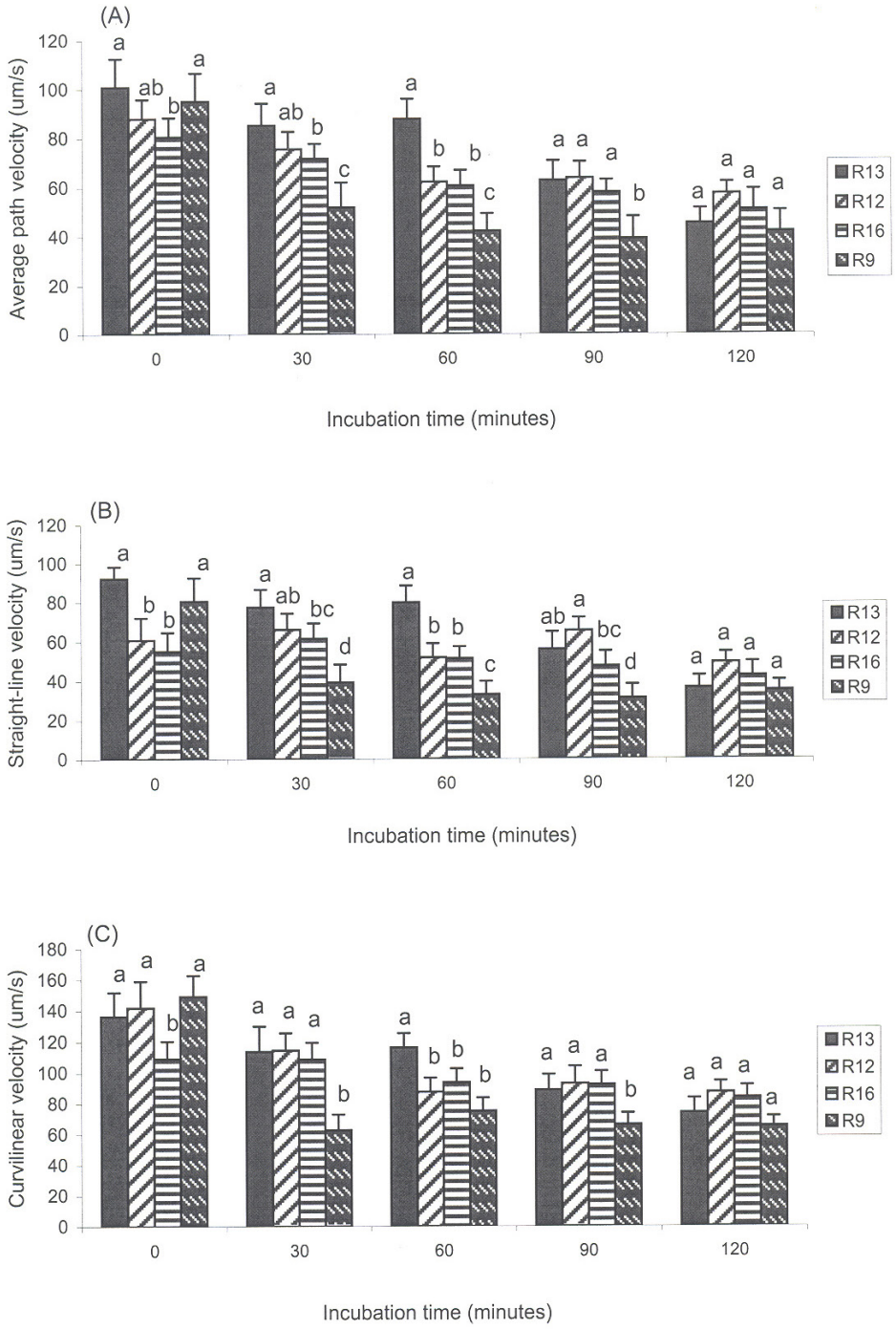

Figure 2 Effect of incubation in TALP medium at 39 ºC on the average path velocity (Figure A), straight-line velocity (Figure B) and curvilinear velocity (Figure C) of spermatozoa from rams (R13, R12, R16, R9) (mean ± SEM). Different letters above bars indicate significant differences ($P \leq 0.05$) within each incubation time.

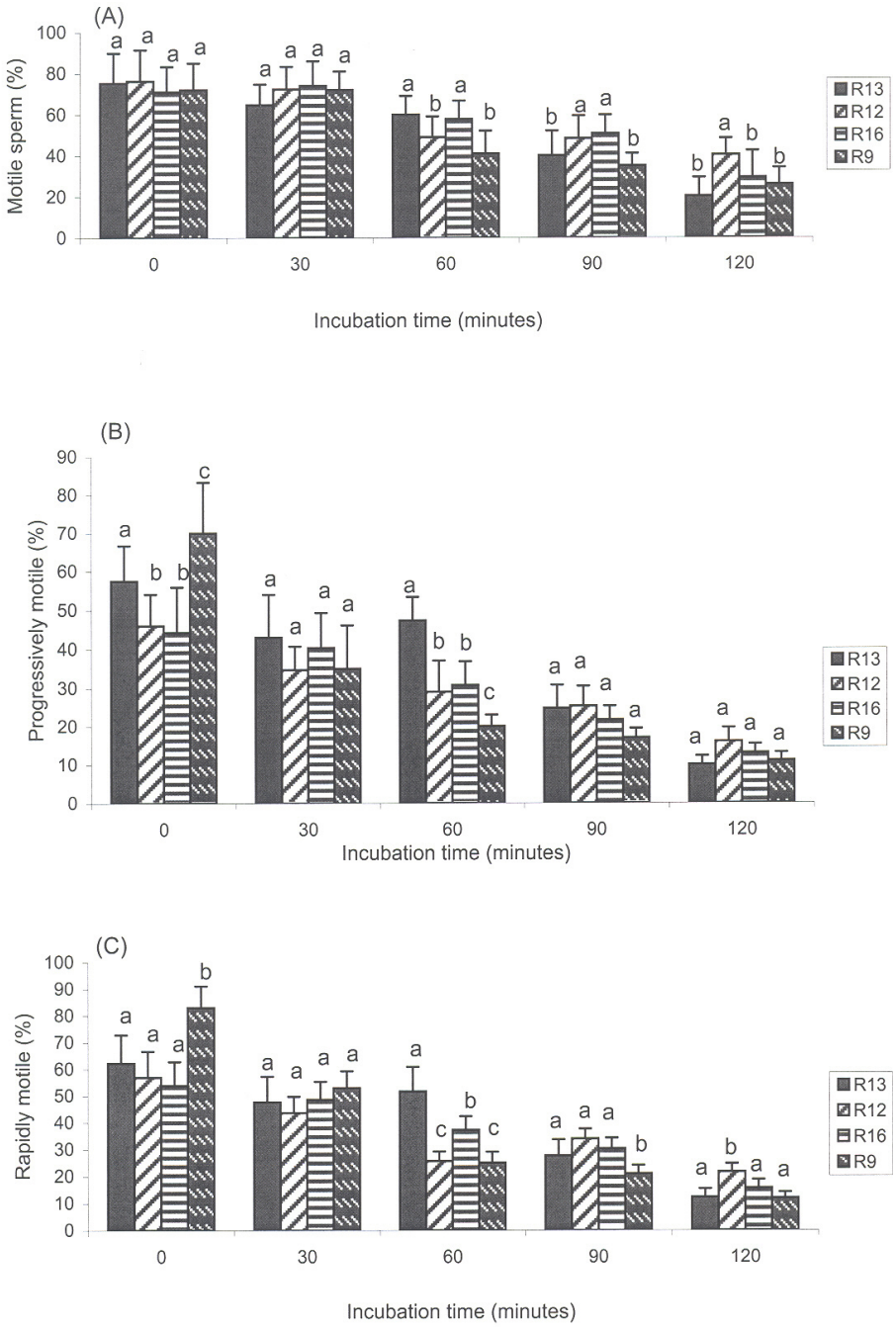

Figure 1 Effect of incubation in TALP medium at 39 ºC on the percentage of motile (Figure A), progressively motile (Figure B) and rapidly motile (Figure C) spermatozoa from rams (R13, R12, R16, R9) (mean ± SEM). Different letters above bars indicate significant differences ($P \leq 0.05$) within each incubation time.