Original Article

Comparative Study on Six Different Long Term Commercial Extenders for Fresh Boar Semen

Kampon Kaeoket* Titisa Srisowanna Ubonrat Wichaidit

Panida Chanapiwat Sukanya Manee-in

Abstract

In Thailand, there are numerous commercial extenders for preservation of boar semen; however, no study has been carried out to compare these extenders in view of maintaining of sperm motility, plasma membrane integrity and acrosome integrity during cold storage. The aim of this study was to assess the percentage of sperm motility, HOST positive, viability and acrosome integrity of boar spermatozoa extended in Merck-III, Androstar®Plus, Modena™, NUTRIXcell®, VITASEM LD, Duragen and Dofu gold™. Ejaculated from boars (n=6, one ejaculate from each boar) were collected and sub-samples (splited samples) were diluted ($3x10^9$ spermatozoa/100 ml) in the different extenders and stored for 10 days at 18°C. On every second day (days 0, 2, 4, 6, 8, 10) after storage, the sperm parameters such as sperm motility, HOST positive, viability and acrosome integrity were evaluated. There were significant differences in characteristic of sperm motility and acrosome integrity between extenders. For instance, on day 4, Merck III (short term extender) and other long term extenders are able to maintain the semen qualities as claimed by the manufacturing. On day 8, the percentages of sperm motility, viability and acrosome integrity were highest ($p< 0.01$) in Androstar®Plus (72%) and Modena™ (75%), Androstar®Plus (67%) and Modena™ (65.6%), and Androstar®Plus (74.6%), Duragen (72.4%) and VITASEM LD (71.6%), respectively. In conclusion, changes in motility, viability and acrosome integrity during storage were affected by the extender utilized, however long term extenders maintained a high percentage (70%) of sperm motility (i.e. Androstar®Plus, Modena™ and Duragen) and acrosome integrity (i.e. Androstar®Plus, VITASEM LD and Duragen) through 8 days of storage.

Keywords: Acrosome integrity, boar, long term extenders, motility, viability

Semen Laboratory, Faculty of Veterinary Science, Mahidol University, Phutthamonthon 4 Rd., Salaya, Phuthamonthon, Nakorn-pathom 73170, Thailand.
Corresponding author E-mail: *vskkk@mahidol.ac.th*

บทคัดย่อ

การศึกษาเปรียบเทียบสารละลายเจือจางน้ำเชื้อแบบระยะยาว 6 ชนิด ในน้ำเชื้อสดของสุกร

กัมพล แก้วเกษ ฐิติสา ศรีโสวรรณา อุบลรัตน์ วิชัยดิษฐ พนิดา ชนาภิวัฒน์*
สุกัญญา มณีอินทร์

ในประเทศไทยมีสารละลายเจือจางน้ำเชื้อสุกรแบบระยะยาวหลายชนิด แต่ยังไม่มีการศึกษาเปรียบเทียบประสิทธิภาพในการรักษาอัตราการเคลื่อนที่ไปข้างหน้า ความสมบูรณ์ของพลาสมาเมมเบรน และความสมบูรณ์ของอะโครโซมของอสุจิ ระหว่างการเก็บรักษา การศึกษาครั้งนี้จึงมีวัตถุประสงค์เพื่อประเมินอัตราการเคลื่อนที่ไปข้างหน้า ความสมบูรณ์ของพลาสมาเมมเบรน และความสมบูรณ์ของอะโครโซมของอสุจิ ในน้ำเชื้อของสุกรที่เจือจางในสารละลาย Merck-III, Androstar®Plus, Modena™, NUTRIXcell ®, VITASEM LD, Duragen และ Dofu gold™ โดยการรีดเก็บน้ำเชื้อจากพ่อสุกรจำนวน 6 ตัว (ตัวละ 1 ครั้ง) หลังจากนั้นแบ่งน้ำเชื้อเป็น 7 ส่วนเพื่อเจือจางในสารละลายเจือจางน้ำเชื้อ 7 ชนิด โดยเตรียมน้ำเชื้อให้ได้ความเข้มข้น $3x10^9$ ตัวต่อสารละลายเจือจาง 100 มิลลิลิตร และเก็บไว้ในตู้เย็นควบคุมอุณหภูมิที่ 18 องศาเซลเซียส จากนั้นประเมินคุณภาพน้ำเชื้อ ในวันที่ 0 (วันที่รีดเก็บ) วันที่ 2 วันที่ 4 วันที่ 6 วันที่ 8 และวันที่ 10 พบว่าหลังจากเก็บน้ำเชื้อไว้นาน 4 วัน Merck III (สารละลายเจือจางน้ำเชื้อแบบระยะสั้น) และสารละลายเจือจางน้ำเชื้อแบบระยะยาวยังสามารถรักษาคุณภาพของน้ำเชื้อได้ตามที่ผู้ผลิตได้กล่าว หลังจากเก็บน้ำเชื้อไว้นาน 8 วัน พบว่า อัตราการเคลื่อนที่ไปข้างหน้า การตรวจความสมบูรณ์ของเมมเบรน ความสมบูรณ์ของพลาสมาเมมเบรน และความสมบูรณ์ของอะโครโซมของอสุจิมีค่าสูงสุด ($p<0.01$) ในสารละลาย Androstar®Plus (72%) และ Modena™ (75%), Androstar®Plus (67%) และ Modena™ (65.6%), และ Androstar®Plus (74.6%), Duragen (72.4%) และ VITASEM LD (71.6%) ตามลำดับ จากผลการทดลองพบว่าการใช้สารละลายเจือจางน้ำเชื้อแต่ละชนิดมีผลต่อการเปลี่ยนแปลงอัตราการเคลื่อนที่ไปข้างหน้า ความสมบูรณ์ของเมมเบรน ความสมบูรณ์ของพลาสมาเมมเบรนและความสมบูรณ์ของอะโครโซมของอสุจิในระหว่างการเก็บรักษา และสารละลายเจือจางน้ำเชื้อแบบระยะยาวมีประสิทธิภาพสูง (70%) ในการรักษาอัตราการเคลื่อนที่ไปข้างหน้า (Androstar®Plus Modena™ และ Duragen) และความสมบูรณ์ของอะโครโซมของอสุจิ(Androstar®Plus VITASEM LD และ Duragen) ในการเก็บรักษาเป็นเวลา 8 วัน

คำสำคัญ: ความสมบูรณ์ของอะโครโซม พ่อสุกร สารละลายเจือจางน้ำเชื้อแบบระยะยาว อัตราการเคลื่อนที่ไปข้างหน้า การตรวจความสมบูรณ์ของเมมเบรน ความสมบูรณ์ของพลาสมาเมมเบรน

ห้องปฏิบัติการน้ำเชื้อ คณะสัตวแพทยศาสตร์ มหาวิทยาลัยมหิดล ถ. พุทธมณฑลสาย 4 ต. ศาลายา อ. พุทธมณฑล จ.นครปฐม 731702
***ผู้รับผิดชอบบทความ E-mail:** vskkk@mahidol.ac.th*

Introduction

In Thailand, the swine industry use artificial insemination over 90% and farmers had paid more attention on monitoring the quality of semen samples on farm or send to semen laboratory at the university, every 3-4 months. Normally, artificial insemination with fresh semen is performed immediately after dilution or keeping for a day at 15-20°C, using a dose of $3-5x10^9$ spermatozoa with a volume of 80-100 ml.

Semen extenders that are available in worldwide, including Thailand are divided into short term extender (such as Beltsville Liquid (BL-1), Beltsville Thawing Solution (BTS), Illinois Variable Temperature (IVT), Merck-III, Kiev and Vital®) and long term extender (such as Acromax, Androhep, Androstar® Plus, Duragen, Dofu gold™, Modena™, MR-A, MULBERRY-III, Reading, NUTRIXcell®, VITASEM LD, Zorlesco and ZORPVA)(Gadea, 2003). Differences between the short term extender and the long term extender is that the latter contains a more complex buffering agents (such as HEPES, Tris, TES and MOPs) and antioxidants (such as bovine serum albumin (BSA), beta-carotene, cysteine, hypotaurine, lycopene, taurine, vitamin E, ascorbic acid, superoxide dismutase and glutathione) (Alvarez and Storey, 1995; Gadea, 2003; Funahashi and Sano, 2005). Antioxidants are well-known in maintaining sperm quality during preservation by diminishing the detrimental effect of reactive oxygen species (ROS) and lipid peroxidation on sperm plasma membrane (Funahashi and Sano, 2005). In general, monitoring of sperm quality composes of macroscopic examination (e.g. evaluation of color, pH and odor) and microscopic examination (e.g. evaluation of morphology, dead/live spermatozoa and

concentration). Semen with high percentage of progressive motility indicates their plasma membrane integrity and excellent metabolism (Johnson et al., 2000) and also reflects their ability for fertilization (Vyt et al., 2004[a,b]; Estienne et al. 2007).

There are many boar semen extenders available in Thailand. However, no study has been carried out to compare the efficacy among those extenders. The aim of the present study was to investigate the efficacy of short term extenders (i.e. Merck-III) and long term extenders (i.e. Androstar®Plus, Modena™, X-Cell®, VITASEM LD, Duragen and Dofu gold) in term of maintaining the sperm motility, functional integrity of plasma membrane (HOST), viability and acrosome integrity.

Materials and Methods

Sperm samples: Six boars (n=6) varied in age (between 2 and 2.5 years) and breed (two Duroc, two Landrace and two Large White). The experimental protocol was approved by The Faculty of Veterinary Science-Animal Care and Use Committee (FVS -ACUC). The boars were housed in individual pens in an opened-house system. Sperm ejaculated from each boar was collected using gloved-hand technique by the same technician (Kaeoket et al., 2008). During collection the semen was filtered though gauze and only sperm rich fraction were collected. Immediately after collection, semen volume, pH using pH meter, semen concentration using SpermaCue™ (Minitübe Abfüll- und Labortechnik GmbH & CO. KG, Tiefenach, Germany), and motility of spermatozoa using phase contrast microscope were evaluated. Hypo-osmotic swelling test (HOST) was determined by hypo-osmotic solution and the slide was examined under 400x magnification of a phase contrast microscope (Chanapiwat et al., 2009). Sperm viability by living cell nucleic acid stain, using SYBR-14, sperm acrosome integrity with fluorescein isothiocyanate-labelled peanut (*Arachis hypogaea*) agglutinin (FITC-PNA) staining and sperm morphology was also evaluated by William staining (Kaeoket et al., 2008). After sperm evaluation, a splited samples design was applied in this study. Semen samples from each boar were diluted to meet a concentration of 3 x 10^9 spermatozoas/100 ml in one short term, i.e. Merck-III® (claimed period of up to 4 days, Minitübe Abfüll- und Labortechnik GmbH & CO. KG, Tiefenach, Germany), and six long term extenders as follows: Androstar®Plus (claimed period of up to 5 days, Minitübe Abfüll-und Labortechnik GmbH & CO. KG, Tiefenach, Germany); Modena™ (claimed period of up to 7 days, Swine Genetics International Ltd., Iowa, USA); NUTRIXcell® (claimed as long term extender, IMV Technologies, Normandy, France); VITASEM LD (claimed period of up to 7-8 days, Magapor S.L., Zaragoza, Spain); Duragen (claimed period of 12-15 days, Magapor S.L., Zaragoza, Spain) and Dofu gold™ (claimed as long term extender, Dofu AI Technology Co., Ltd., Shanghai, China). Thereafter, the diluted boar semen samples in different extenders were further subdivided into different groups according to the types of extenders and day of testing (i.e. 7 extenders and 6 days). Lastly, fresh extended boar semen samples were transported to laboratory in an isothermal box. During the experiment, diluted semen samples were stored at 18°C for 10 days in a refrigerator (digital controlling system, Magapor®, Zaragoza, Spain). Semen evaluation was performed on every second day (days 0, 2, 4, 6, 8, 10) after storage.

Parameters of comparison: Four different parameters were used to evaluate the extended fresh boar semen samples, i.e., percentage of sperm motility, viability, acrosome integrity and HOST. All parameters were determined on every second day (days 0, 2, 4, 6, 8, 10) after storage.

Sperm progressive motility: Sperm progressive motility was assessed by visual estimation by the same person throughout the experiment, under phase contrast microscope at 100x and 400x magnification. Progressive motility was revealed as the percentage of motile spermatozoa (Kaeoket et al., 2008).

Sperm viability (Sperm plasma membrane integrity): The viability was evaluated with SYBR-14/Ethidium homodimer-1. 10 µl of diluted spermatozoa were mixed with 2.7 µl of the user solution of SYBR-14 and 10 µl of EthD-1. After incubation at 37°C for 15 min, a total of 200 spermatozoa were assessed (400x) under fluorescence microscope. The nuclei of spermatozoa with intact plasma membrane stained green with SYBR-14, while those with damaged membranes stained red with EthD-1. Spermatozoa were classified into three types as follows: having intact plasma membrane (stained with green, live); having damaged plasma membrane but an intact acrosome (stained with red and green, damaged) and having both plasma membrane and acrosome damaged stained with red, namely dead (Kasetrtut and Kaeoket, 2010c). In the results, percentage of live, damaged and dead spermatozoa are presented.

Sperm acrosome integrity: Acrosome integrity was evaluated using fluorescein isothiocyanate–labeled peanut (*Arachis hypogaea*) agglutinin (FITC-PNA) staining. Ten µl of the diluted semen was mixed with 10 µl of Ethidiumhomodimer-1 and incubated at 37°C for 15 min. Five µl of the mixture was smeared on a glass slide and fixed with 95% ethanol for 30 sec. Fifty µl Fit C-PNA (dilute Fit C-PNA with PBS 1:10 v/v) was spread over the slide and incubated in a moist chamber at 4°C for 30 min. After incubation, it was rinsed with cold PBS and air dried. Two hundred spermatozoa were assessed under fluorescent microscope at 1000x magnification and classified as intact acrosome, damaged acrosome and missing acrosome (Maxwell and Johnson, 1997; Chanapiwat et al., 2009; Kaeoket et al., 2010[b]). The results are scored as the percentage of intact acrosome spermatozoa.

The functional integrity of the sperm plasma membrane: The functional integrity of the sperm plasma membrane was assessed using a short hypo-osmotic swelling test (HOST) (Chanapiwat et al., 2009). Spermatozoa were incubated, at 38°C for 30 min, with 75 mOsm/kg a hypo-osmotic solution that consist of 0.368% (w/v) Na-citrate and 0.675% (w/v) fructose (Merck, Germany) in distilled water. Following this incubation time, 200 µl of the semen-hypo-osmotic solution was fixed in 1000 µl of a hypo-

osmotic solution plus 5% formaldehyde (Merck, Germany), for later evaluation. Two hundred spermatozoa were assessed under a phase contrast microscope at 400x magnification. The coiled tail (HOST positive) spermatozoa found following incubation were functional intact plasma membrane.

Statistical analyses: The statistical analysis was made using the Statistical Analysis System software package (Version 9.0, SAS Institute Inc., 1996, Cary, N.C., USA). Percentages of sperm motility, viability, acrosome integrity and HOST were analyzed using the General Linear Mixed Model (MIXED) procedure of SAS. The model include group of extender as a fixed effect and include boar as random effects. Statistically significant difference was defined as $p< 0.05$.

Results

Average pH and osmolarity of each commercial extender before and after dilution are shown in Tables 1, 2 and 3, respectively. The semen quality parameters included sperm motility, viability, acrosome integrity and HOST in the different extenders after storage at 18°C are presented in Tables 4-9.

Motility: After 48 hours of storage, there were no statistically significant differences in all parameters among extenders. On day 4, for long term extender, the highest progressive motility was found in Modena™ (86%), Androstar®Plus (84%), Duragen (83%) and VITASEM LD (80%). For short term extender (Merck-III), the progressive motility is close to 70%. On day 6, for long term extender, Modena™ and Duragen showed progressive motility of higher than 80%. The Androstar®Plus showed progressive motility of higher than 70%. Merck-III showed progressive motility as low as 50%. On day 8, there are 3 long term extenders, consist of Modena™, Androstar®Plus and Duragen that can maintain progressive motility of more than 70%. The lowest motility was found in semen diluted with Merck-III. On day 10, none of extenders can maintain motility of more than 70%. Androstar®Plus (68%) and Modena™ (68%) showed motility close to 70%.

Acrosome integrity: From days 0-4, both short term and long term extenders can maintain acrosome integrity of more than 70%. On day 6, the highest acrosome integrity was found in semen extended with VITASEM LD (75%) and Androstar®Plus (74%). Dofu gold™ (67%) and Merck-III (66%) showed progressive motility of less than 70%. On day 8, Androstar®Plus (74%), Duragen (72%) and VITASEM LD (70%) can maintain acrosome integrity of more than 70%. On day 10, only VITASEM LD showed acrosome integrity of more than 70%.

Hypoosmotic swelling test (HOST) and viability: On days 4 and 6, only Modena™ (73%) showed viability of more than 70%. The lowest viability was found in semen diluted with Merck-III. On day 8, for long term extender, Androstar®Plus (67%), Modena™ (65%) and Dofu gold™ (60%) showed viability of more than 60%. On day 10, VITASEM LD (60%) was only one that showed viability of more than 60%. There was no significant different among extenders in term of hypoosmotic swelling test (HOST). The percentage of HOST positive varied between 12 and 28%.

Table 1. pH and osmolarity of each commercial extenders (mean±SD)

Parameters (n=3)	Merck-III	Androstar®Plus	Modena™	X-Cell®	Dofu gold™	VITASEM LD	Duragen
pH	7.2±0.3	7.2±0,2	7.1±0.1	7.4±0.2	6.9±0.2	7.4±0.3	7.2±0.2
Osmolarity	360±5.8	294±4.6	293±5.6	316±2.4	290±3.1	293±5.2	297±4.1

Table 2. pH of each commercial extenders after diluted with fresh semen (mean±SD)

Day (n=6)	Merck-III	Androstar®Plus	Modena™	X-Cell®	Dofu gold™	VITASEM LD	Duragen
0	7.3±0.1	7.3±0.1	7.1±0.2	7.4±0.1	7.0±0.2	7.4±0.1	7.4±0.1
2	7.0±0.1	7.0±0.2	6.8±0.1	7.7±0.1	6.8±0.1	7.0±0.2	7.0±0.1
4	6.8±0.2	6.8±0.1	6.7±0.1	6.8±0.2	6.6±0.1	6.8±0.1	6.7±0.1
6	6.8±0.2	6.7±0.1	6.6±0.1	6.8±0.2	6.6±0.1	6.8±0.1	6.7±0.2
8	6.8±0.2	6.6±0.1	6.5±0.1	6.8±0.3	6.5±0.1	6.7±0.1	6.6±0.1
10	6.8±0.2	6.5±0.1	6.5±0.1	6.5±0.1	6.4±0.0	6.6±0.1	6.5±0.1

Table 3. Osmolarity of each commercial extenders after diluted with fresh semen (mean±SD)

Day (n=6)	Merck-III	Androstar®Plus	Modena™	X-Cell®	Dofu gold™	VITASEM LD	Duragen
0	354.5±6.2	297.5±6.5	292.8±5.5	313±4.4	292.5±11.2	296±4.4	294.8±6.5
2	353.6±4.7	297±5.9	297.8±5.6	312±2.9	292.6±3.7	296±3.4	296.5±3.7
4	353.5±9.1	296.5±6.9	298.1±8.2	314±4.8	293.3±5.1	299.3±8.9	295±4.5
6	354±5.3	299±5.4	296.8±5.6	312.5±4.4	293.6±1.4	297.6±5.1	296.6±4.8
8	352.3±6.2	297.5±8.0	296.8±5.8	313±3.2	295.8±3.5	298.1±6.3	297.3±2.5
10	353.8±4.8	298.5±5.4	299.3±4.4	315.4±1.5	299.3±7.9	304.5±7.6	298.1±3.4

Table 4. Progressive sperm motility, HOST, viability and acrosome integrity on day 0 (mean±SD)

Parameters	Merck-III	Androstar®Plus	Modena	X-Cell®	Dofu gold	VITASEM LD	Duragen
Progressive motility,%	87.0±6.7	89.0±5.5	92.0±2.7	66.0±11.4	87.0±7.6	87.0±10.4	92.0±6.7
HOST	23.1±14.3	20.6±8.3	18.5±9.6	18.5±11.0	20.4±10.9	18.7±10.6	20.0±10.3
Viability,%	74.4±5.9	77.7±13.3	78.7±8.3	67.0±16.7	79.9±10.6	77.4±5.7	79.0±8.4
Acrosome integrity,%	81.0±6.3	97.2±8.9	84.6±3.1	77.8±11.0	83.8±5.2	79.6±6.7	81.2±8.2

Table 5. Progressive sperm motility, HOST, viability and acrosome integrity on day 2 (mean±SD)

Parameters	Merck-III	Androstar®Plus	Modena™	X-Cell®	Dofu gold™	VITASEM LD	Duragen
Progressive motility,%	82.0±3.4^{a}	83.0±6.5^{a,b}	89.0±5.5^{a,b}	72.0±8.4^{a,b}	86.0±10.8^{b}	85.0±10.6^{a,b}	87.0±9.7^{a,b}
HOST	25.7±12.0	23.3±3.8	23.9±6.9	17.5±2.8	21.3±6.0	20.9±10.1	18.3±4.0
Viability,%	64.6±13.0	70.8±3.9	74.8±8.5	64.3±5.8	73.3±11.5	72.8±5.8	69.1±11.2
Acrosome integrity,%	78.6±4.7	76.8±5.8	81.8±4.1	72.0±4.6	79.2±4.5	81.0±4.1	80.4±6.3

Values followed by different alphabets within the same row against each parameter were significantly different (p<0.01)

Table 6. Progressive sperm motility, HOST, viability and acrosome integrity on day 4 (mean±SD)

Parameters	Merck-III	Androstar®Plus	Modena™	X-Cell®	Dofu gold™	VITASEM LD	Duragen
Progressive motility,%	68.0±13.0^{a,b}	84.0±4.2^{a,b}	86.0±4.2^{b}	64.0±8.9^{a}	78.0±8.4^{a,b}	80.0±7.1^{a,b}	83.0±4.5^{a,b}
HOST	25.9±8.4	27.3±7.9	26.6±7.8	19.0±5.8	27.7±10.2	24.7±4.7	25.8±5.6
Viability,%	55.8±21.1	65.3±10.9	72.3±13.9	58.7±7.2	65.1±11.9	65.2±7.2	68.8±11.1
Acrosome integrity,%	73.2±7.9	74.6±13.8	74.0±13.0	77.4±9.4	78.4±7.3	75.2±7.0	77.2±10.7

Values followed by different alphabets within the same row against each parameter were significantly different (p<0.01)

Table 7. Progressive sperm motility, HOST, viability and acrosome integrity on day 6 (mean±SD)

Parameters	Merck-III	Androstar®Plus	Modena™	X-Cell®	Dofu gold™	VITASEM LD	Duragen
Progressive motility,%	50.0±7.0^{b}	77.0±6.7^{a,b}	82.0±2.7^{a}	68.0±11.0^{a,b}	68.0±13.0^{a,b}	72.0±13.0^{a}	80.0±10.0^{a}
HOST	14.9±3.4	16.4±3.6	15.9±3.7	14.8±5.3	18.2±3.3	14.0±3.6	12.5±4.4
Viability,%	57.4±4.2	65.8±3.0	73.5±6.0	59.9±6.2	65.1±9.0	69.3±8.1	68.8±9.6
Acrosome integrity,%	66.0±2.2	74.6±8.0	73.2±6.1	71.0±3.6	67.2±13.3	75.4±8.5	72.6±7.5

Values followed by different alphabets within the same row against each parameter were significantly different (p<0.01)

Table 8. Progressive sperm motility, HOST, viability and acrosome integrity on day 8 (mean±SD)

Parameters	Merck-III	Androstar®Plus	Modena™	X-Cell®	Dofu gold™	VITASEM LD	Duragen
Progressive motility,%	34.0±18.2^{c}	72.0±8.4^{a,b}	75.0±7.1^{a}	52.0±8.4^{b,c}	54.0±8.9^{a,b,c}	69.0±12.4^{a,b}	70.0±12.2^{a,b}
HOST	16.5±7.1	20.3±7.0	19.8±4.7	14.2±3.1	18.8±6.2	20.6±13.7	15.1±3.0
Viability,%	52.5±9.0	67.0±7.4	65.6±9.4	52.8±8.3	60.2±13.7	59.7±9.8	59.0±10.0
Acrosome integrity,%	67.5±6.6	74.6±2.6	68.0±10.4	68.0±12.0	65.2±11.3	71.6±10.6	72.4±6.6

Values followed by different alphabets within the same row against each parameter were significantly different (p<0.01)

Table 9. Progressive sperm motility, HOST, viability and acrosome integrity on day 10 (mean±SD)

Parameters	Merck-III	Androstar®Plus	Modena™	X-Cell®	Dofu gold™	VITASEM LD	Duragen
Progressive motility,%	14.0±11.4^{b}	68.0±8.4^{a,c}	68.0±13.0^{a,c}	36.0±16.7^{b}	34.0±16.7^{b}	60.0±21.2^{a,c}	64.0±15.2^{a,c}
HOST	12.5±6.4	20.8±4.9	27.5±18.4	16.5±9.4	18.0±4.4	20.7±5.3	14.8±3.6
Viability,%	42.5±2.6	58.1±6.6	58.8±4.5	43.3±8.7	57.3±6.4	60.4±8.7	50.3±9.6
Acrosome integrity,%	61.5±6.2	69.1±11.3	66.1±4.6	63.8±2.9	67.2±4.9	70.4±4.8	62.8±6.7

Values followed by different alphabets within the same row against each parameter were significantly different (p<0.01)

Discussion

In this study, we compared seven extenders with regard to motility, HOST, viability and acrosome integrity of boar spermatozoa during storage for 10 days at 18°C. The functional integrity of the sperm plasma membrane, viability and acrosome integrity of boar spermatozoa did not vary significantly during the examined period. Most of the long term extenders are maintained the semen qualities up to 7 days as claimed by a manufacturer. Attractively, short term extenders used in this study, i.e. Merck-III with declared preservation period of 3 days showed capability to maintain sperm quality that was not considerably different from long term extenders during cold storage for 4 days. Merck-III was supplemented with an antioxidant, namely taurine. Taurine (2-aminoethanesulfonic acid) is the major intracellular free amino acid, which plays an important role in osmotic pressure control and minimizes the detrimental effect of oxidant-induced tissues damage, consequently improve viability of sperm cells (Yang et al., 2006). It has been shown in other species, e.g. stallion (Ijaz and Ducharme, 1995), ram (Uysal and Bucak, 2007), bull (Sariozkan et al., 2009), including human (Holmes et al., 1992) that taurine is a superior alternative antioxidant when preservation of semen is considered necessary. The profitable effects of taurine as an antioxidant are to stabilize plasma membranes, scavenged the reactive oxygen species and reduced the production of lipid peroxidation (Yang et al. 2006; Sariozkan et al. 2009). These may explain the fact that Merck-III is able to maintain sperm longevity as long as 4 days in the present study.

Motility is an important parameter for evaluating the boar semen quality because the movement of spermatozoa indicates active metabolism and integrity of membranes, and also importance for fertilization (Vyt et al., 2004[a,b]; Estienne et al., 2007). In this study, motility was depending on time of storage. In Merck-III, spermatozoa showed a satisfied motility after storage for 4 days, although there was a subsequently decline afterward. During long term storage in low temperature (18°C), spermatozoa are changes in motility, viability and alterations membrane integrity. These can be explained by the fact that sperm have a high content of unsaturated fatty acids in their membranes (Johnson et al., 1969; Park and Lynch, 1992) and they lack a significant cytoplasmic component containing antioxidants (de Lamirande and Gagnon, 1995; Saleh and Agarwal, 2002). Therefore, sperms are highly susceptible to lipid peroxidation (LPO), which leads to sperm membranes damage during preservation (Sikka et al., 1995). In addition, it has been shown that cold shock of sperm during preservation is associated with oxidative stress induced by free radicals (i.e. reactive oxygen species, ROS) (Uysal and Bucak, 2007).

Excessive ROS formation by spermatozoa during preservation has been associated with damaging plasma membrane and consequently reducing sperm motility (Kumaresan et al., 2009). In addition, lipid peroxidation during cold storage can also damage sperm plasma membrane, inhibit of respiration and intracellular enzymes leakage (White, 1993). When the lipid peroxidation is encouraged, 60% of fatty acid is lost from the membrane, subsequently loss of sperm membrane integrity (Jones et al., 1979). To reduce the detrimental effects of ROS on sperm quality, supplementation antioxidants in the extender in order to protect sperm plasma membrane from oxidative stress has been studied. Funahashi and Sano (2005) reported that the supplementation extended fresh boar semen with GSSG, BSA, *L*-cysteine and lycopene improved boar semen quality during cold storage. Besides, it has also been shown that addition of some antioxidants, such as water soluble Vitamin E, glutathione, *L*-cysteine to semen extenders for freezing boar spermatozoa improved post-thawed sperm motility and viability (Kaeoket et al., 2008; Chanapiwat, 2009; Kaeoket et al., 2010[a]).

Our results showed that long term extenders which comprises of some antioxidants are able to maintain sperm motility as long as 8 days. This might be due to the direct effect of antioxidants in term of inhibiting the lipid peroxidation and diminishing the quantity of ROS during the cold storage. To our knowledge, Modena™ and Androstar®Plus are supplemented with antioxidant, known as L-cysteine which has important role in membrane stabiliser and capacitation inhibitor, and also bovine serum albumin (BSA) which play a critical role in protection against cold shock and inhibit lipid peroxidation (Alvarez and Storey, 1995; Gadea, 2003). In addition, these long term extenders contain a potential chelating agent (i.e. EDTA) which has a significant function to block the action of calcium as a mediator of sperm capacitation and the acrosome reaction during cold storage. Besides the supplementation of antioxidants and chelating agents, the buffering agent (e.g. Hepes and Tris) was also added in the extender in order to regulate the pH of this particular extender. Hepes in Androstar ®Plus and Tris in Modena™ are more complex buffers agents in which can regulate the pH over a wider range and not depend on the temperature (Gadea, 2003). However, for the other long term extenders, their compositions are still a commercial secret.

Considering short term extender and long term extender, there are not differ from each other in term of energy source (i.e. glucose, galactose, fructose, ribose or trehalose), in which these sugar improved cell metabolism and the movement of the flagellum via glycocilic pathway, the buffering agents (i.e. bicarbonate and sodium citrate) and also the osmotic pressure regulators (i.e. potassium chloride and sodium chloride) (Gadea, 2003).

In conclusion, changes in motility, HOST, viability and acrosome integrity during storage were affected by the extender utilized. In addition, long term extenders (i.e. Androstar®Plus, Modena™ and Duragen) maintained a satisfied motility (70%) through 8 days of storage.

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