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Study on the *Marsilea crenata* extract supplementation on caudal epididymal plasma (CEP-3) to the Boer semen quality during cold storage

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ABSTRACT: The success of artificial insemination (AI) would be determined by the semen quality during storage. This research aims to evaluate the quality of Boer goat liquid semen stored with caudal epididymal plasma (CEP-3) supplemented with water clover (*Marsilea crenata*) extract or WCE during cold storage (3-5℃). The semen was collected from Boer goat aged 2.5 to 3 years old by using an artificial vagina. The semen with >2+ mass motility and >70% individual motility were then selected for the study. A randomized block design which consisted of four treatments with ten replications as follow: P_0 or control (CEP-3 + 10% Egg Yolk + 0,4% Albumen); P_1 (CEP-3 + 10% egg yolk + 0.4% albumen + 1% WCE; P_2 (CEP-3 + 10% egg yolk + 0.4% albumen + 2% WCE); P_3 (CEP-3 + 10% egg yolk + 0.4% albumen + 3% WCE) was used in this study. The data were analyzed with ANOVA and followed with Duncan's multiple range test to determine significant differences. The results of this study showed that motility, viability, and membrane integrity in all treatments showed highly significant differences ($P<0.01$), while no significant difference ($P>0.05$) was not shown on the semen abnormality. The result concludes that 1% WCE supplementation to the CEP-3 extender showed the best Boer semen quality during 96-hours cold storage.

Keywords CEP-3; antioxidant; semen quality; water clover extract.

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INTRODUCTION

Goats are potential livestock to be developed as meat and milk producers to fulfill the increasing demand for animal-based protein. The population of goats in Indonesia has been fluctuating, which had increased as much as 373,261 goats in 2015, and then decreased as much as 1,165,597 goats in 2016 and 563,182 goats in 2017 (Ditjennak, 2017). Artificial insemination technology is then introduced to increase livestock productivity, population, and genetic quality.

One of the factors which influence the success of artificial insemination is the semen quality. The liquid semen can be used to improve the success of artificial insemination aside from using frozen semen. The artificial insemination by using liquid semen at 5-days storage is relatively higher compared to frozen semen at 5-days storage in terms of value conception rate (Susilawati *et al*., 2016).

The semen extender is a material that could be added into liquid semen to support the life of spermatozoa. The semen extender should fulfill certain requirements to maintain the spermatozoa quality, such as the ability to provide energy source or nutrition, maintain the semen pH at around 6.8 – 7 and inhibit cold shock (Feradis, 2010). The CEP-2 extender is one of the semen extenders developed by Verberckmous *et al*. (2004), which imitates plasma composition produced in cauda epididymis by male reproductive tracts. However, the CEP-2 extender required bovine serum albumin (BSA) for its application, while also relatively expensive as well. However, the application of BSA can be substituted with egg white to produce CEP-3 extender (Istanty *et al*., 2017). The semen storage in low temperatures could prolong the semen quality, but peroxidation by free radicals could occur during long-time storage. The addition of antioxidants is then suggested to inhibit the reaction.

Water clover (*Marsilea crenata*) is a group of aquatic fern (*Hydropterides*) which contains phytochemical compounds and antioxidants. The crude extract of water clover by using methanol has been known to contain antioxidant compounds in the form of flavonoids (Nurjannah *et al*., 2012). Flavonoid is an antioxidant with good free radical acceptor capabilities (Sudarmanto and Suhartati, 2015). The antioxidant content in water clover extract (WCE) is then showed its potential to be added in the semen extender to inhibit peroxidation during storage. This study is then conducted to determine the effect of WCE supplementation on CEP-3 to the Boar goats' semen quality during cold storage.

METHOD OF THE RESEARCH

The materials of this study were Boer goat semen collected from 3 Boer goats aged at 2.5-3 years old reared in Sumbersekar farm laboratory. The semen was collected by using artificial vagina and the semen with >2+ mass motility and >70% individual motility was used for this study. The semen was then diluted by using CEP-3 extender with the addition of water clover (*Marsilea crenata*) extract or WCE at different concentration levels (0%, 1%, 2%, 3%). The water clover extraction was conducted at the UPT (Technical Implementation Unit) Materia Medica Batu Malang by methanol extraction method.

The study is conducted as an experimental research with Randomized Block Design consisted of 4 treatments and 10 replications. The observation was done at 0, 12, 24, 48 and 96 hours, and the obtained data were analyzed by analysis of variance (ANOVA) followed with Duncan's multiple range test to determine significant differences. The treatments in this study were as follow:

P0: 90% CEP-3 diluter + 10% egg yolk + 0.4% egg white + 0% WCE
P1: 90% CEP-3 diluter + 10% egg yolk+ 0.4% egg white + 1% WCE

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P2: 90% CEP-3 diluter + 10% egg yolk + 0.4% egg white + 2% WCE
P3: 90% CEP-3 diluter + 10% egg yolk + 0.4% egg white + 3% WCE

The semen treatments were then storage at 3-5ºC and observed on its individual motility, viability, abnormality, and membrane integrity by following Ducha *et al*., (2012) and Lukman *et al*., (2014).

RESULTS AND DISCUSSION

Individual Motility during Cold Storage

The spermatozoa quality can be seen from its progressive motilities. The individual spermatozoa motility observation on the Boer goat semen in this study is presented in Table 1.

Table 1. The Percentage of Individual Spermatozoa Motility in Cold Storage at 3-5ºC

Time (hours)	Individual Motility (%)			
	P0	P1	P2	P3
0	65.50 ± 4.97^{a}	71.50 ± 4.74^{b}	67.50 ± 4.25^{a}	65.00 ± 3.33^{a}
12	60.00 ± 5.27^{a}	66.50 ± 4.74^{b}	62.50 ± 4.25^{ab}	59.50 ± 2.84^{a}
24	54.50 ± 5.50^{a}	61.50 ± 4.74^{b}	57.50 ± 4.25^{ab}	54.50 ± 2.84^{a}
48	49.00 ± 6.15^{a}	56.50 ± 4.74^{b}	52.50 ± 4.25^{ab}	49.50 ± 2.84^{a}
96	43.50 ± 6.69^{ab}	51.50 ± 4.74^{b}	47.50 ± 4.25^{ab}	39.50 ± 3.16^{a}

Different superscripts showed highly significant differences (P <0.01)

During cold storage, the decrease in individual spermatozoa motility has occurred. The condition is caused by the produced lactic acid by metabolic process of the spermatozoa. The semen cold storage at 3-5ºC is aimed to inhibit the metabolic process. However, during this cold storage, lipid peroxidation was occurred and will reduce the spermatozoa motility through free radical production. Daniel *et al*. (2010), showed that free radicals are metabolic products that frequently produced by cells. Feradis (2010) explained that lipid peroxidation reaction occurred by the continuous chain reaction of free radicals and unsaturated fatty acids in spermatozoa. The produced free radicals would be more reactive and damage the cell (Latief *et al.*, 2013).

The analysis of variance on the spermatozoa individual motility showed a highly significant difference ($P<0.01$) during the 0-48 hours cold storage between 1% WCE supplementation and other treatments. Moreover, the 1% WCE (P_1) supplementation showed a highly significant effect on the individual spermatozoa motility compared to the P_2 and P_3 as well. The condition thus indicates that the higher WCE supplementation could be toxic for the spermatozoa and reduce its motility. The 1% WCE supplementation thus could provide enough antioxidants to maintain spermatozoa motility. Antioxidants tend to react with free radicals as the compounds are easily oxidized and had strong reducing capabilities (Khaira, 2010). Crude water clover extract contains antioxidants in the form of flavonoids that can inhibit cell damage by reacting with free radicals (Latief *et al.*, 2013).

Spermatozoa Viability during Cold Storage

Spermatozoa viability is one of the determining parameters in the semen quality. The spermatozoa viability during cold storage in this study is presented in Table 2.

Table 2. The percentage viability of spermatozoa in cold storage

Duration (hours)	Spermatozoa viability (%)			
	P0	P1	P2	P3
0	82.84 ± 7.94[a]	86.07 ± 4.53[a]	82.17 ± 7.78[a]	82.52 ± 8.18[a]
12	77.07 ± 6.05[a]	83.36 ± 6.26[b]	78.34 ± 8.85[ab]	73.50 ± 9.22[a]
24	72.55 ± 7.89[ab]	79.52 ± 7.34[b]	73.39 ± 8.08[ab]	69.37 ± 9.18[a]
48	62.68 ± 8.88[ab]	74.96 ± 8.63[b]	67.22 ± 9.47[ab]	56.84 ± 14.40[a]
96	52.60 ± 5.55[a]	67.08 ± 6.26[b]	59.00 ± 5.78[ab]	46.73 ± 17.83[a]

Different superscripts showed highly significant differences ($P < 0.01$)

The results showed that the spermatozoa viability showed significant differences ($P<0.01$) during 12 and 96-hours cold storage between P_1 and other treatments. The decrease of individual motility is in line with spermatozoa viability. The decreased spermatozoa viability is caused by the lactic acid accumulation produced by spermatozoa metabolism. The accumulation thus decreased the semen pH and spermatozoa viability as well. Pereira *et al.* (2010) showed that decreased spermatozoa viability could be caused by low temperature, low nutrients, low pH, and the damaged plasma membrane as well.

The 1% WCE supplementation showed higher spermatozoa viability compared to other treatments, this is caused by the antioxidants contained in the WCE which would maintain the spermatozoa. The right antioxidant supplementation would reduce the free radical production in the semen. The free radical inhibition by antioxidants could occur in several ways, one of the ways is through proton donor mechanism as done by flavonoids in water clover extract (Saefudin *et al*., 2013).

Spermatozoa Abnormalities during Cold Storage

Spermatozoa abnormality is a condition where the spermatozoa are damaged or any spermatozoa abnormalities existed in the sperm cell. The abnormality percentage of spermatozoa in this study is presented in Table 3.

Table 3. The Percentage abnormality of spermatozoa in cold storage

Duration (hours)	Spermatozoa abnormality (%)			
	P0	P1	P2	P3
0	5.32 ± 1.11[a]	5.01 ± 1.26[a]	5.04 ± 1.42[a]	5.01 ± 1.12[a]
12	5.08 ± 1.53[a]	5.39 ± 1.84[a]	5.38 ± 1.38[a]	5.99 ± 1.57[a]
24	5.92 ± 1.22[a]	5.65 ± 2.35[a]	5.51 ± 1.31[a]	5.68 ± 2.11[a]
48	5.96 ± 0.38[a]	5.56 ± 1.93[a]	5.74 ± 1.21[a]	5.78 ± 1.86[a]
96	5.99 ± 1.67[a]	5.74 ± 2.53[a]	5.75 ± 1.69[a]	5.94 ± 3.08[a]

The analysis of variance showed that the WCE supplementation did not give significant effect ($P> 0.05$) to the spermatozoa abnormalities in all treatments. Spermatozoa cells can be damaged during dilution and storage and tend to increase the abnormalities in spermatozoa. Suyadi *et al.* (2017), stated that temperature change during semen dilution can disrupt membrane cell permeability, and increase spermatozoa abnormalities. During the storage, free radicals known as reactive oxygen species (ROS) would be produced, this condition could damage the plasma membrane and increase the spermatozoa abnormalities. All treatments in this study

showed that less than 15% of the spermatozoa were abnormal, thus indicates a good spermatozoa condition. Rochim *et al*. (2017), stated that less than 20% spermatozoa abnormalities are still considered good and can be used for artificial insemination. Ax *et al*. (2008) added that if more than 20% spermatozoa abnormalities would have resulted in low fertility.

Spermatozoa Membrane Integrity during Cold Storage

The plasma membrane integrity would determine spermatozoa life (Lubis *et al*., 2013). The circular tail of spermatozoa would indicate good membrane integrity, while the straight tail would indicate damaged plasma membranes of the spermatozoa. The plasma membrane integrity in this study is presented in Table 4.

Table 4. The percentage of spermatozoa plasma membrane integrity in cold storage

Duration (hours)	Plasma membrane integrity (%)			
	P0	P1	P2	P3
0	68.16 ± 6.32^{a}	75.39 ± 2.89^{b}	72.65 ± 4.76^{ab}	73.75 ± 8.10^{ab}
12	65.43 ± 5.73^{a}	72.22 ± 5.44^{b}	68.36 ± 4.20^{ab}	68.49 ± 4.38^{ab}
24	59.16 ± 6.72^{a}	65.80 ± 3.68^{b}	61.44 ± 5.75^{ab}	65.19 ± 5.93^{b}
48	51.75 ± 7.39^{a}	62.21 ± 4.67^{b}	54.83 ± 6.73^{a}	55.64 ± 5.25^{ab}
96	44.20 ± 11.96^{a}	55.79 ± 8.48^{b}	50.04 ± 7.71^{ab}	46.00 ± 8.34^{a}

Different superscripts showed highly significant differences (P <0.01)

The analysis of variance showed significant differences (P<0.01) of the plasma membrane integrity in all treatments during 96-hours cold storage, with the best plasma membrane integrity, which was shown in 1% WCE supplementation. The higher plasma membrane integrity on 1% WCE supplementation is due to the suitable antioxidant content in the WCE which would inhibit oxidation during cold storage. Feradis (2010) explained that antioxidants would inhibit free radical reactions. In addition, adequate antioxidants would prevent cold shock which would damage the plasma membrane.

During cold storage, the physiological function of the membrane was decreased due to the depolarization of the spermatozoa membrane molecules. Costa *et al*. (2016), showed that CEP-2 and egg yolk is adequate to maintain spermatozoa quality for 6 days. The WCE supplementation in CEP-3 extender added with egg yolks at the right concentration could increase spermatozoa ability to maintain its membrane integrity compared to without any WCE supplementation due to the antioxidant compounds in the extract which inhibit lipid peroxidation.

CONCLUSION

The different Boer goat semen quality during cold storage was found on WCE supplementation in CEP-3 extender compared to without supplementation. The 1% WCE supplementation in CEP-3 extender showed the best semen quality during 96-hours cold storage in terms of spermatozoa motility, viability, and membrane integrity.

REFERENCES

Ax, R., M. Dally., B. Didion., R. Lenz., C. Love., D. Varner., E.S.E. Hafez. and M. Bellin. 2008. *Semen Evaluation. In Reproduction in Farm Animal*. Hafez, E.S.E. (Ed). 7th Edition. Lea and Febiger, USA.

Costa, N., T. Susilawati., N. Isnaini. dan M. Ihsan. 2016. Effect of Different Dilution Materials Usage on Indonesian Peranakan Ongole Bull

Sperm Quality During Cooling Process. *Indo American Journal of Pharmaceutical Sciences*. 3 (4): 379-385.

Daniel, R.M., Stelian, dan Dragomir. 2010. The Effect of Acute Physical Exercise on The Antioxidant Status of The Skeletal and Cardiac Muscle in The Wistar Rat. *Romanian Biotechnological Letters*. 15 (3): 56-61.

Ditjennak. 2017. Statistik Peternakan dan Kesehatan Hewan 2017. Direktorat Jenderal Peternakan dan Kesehatan Hewan Kementerian Pertanian RI. ISBN: 978-979-628-034-6.

Ducha N, T. Susilawati, Aulanniam, S. Wahyuningsih, M. Pangestu. 2012 Ultrastructure and Fertilizing Ability of Limousin Bull Sperm after storage in Cep-2 extender with and without Egg Yolk. *Pakistan Journal of Biological Sciences*, 15 (20):979-985.

Feradis. 2010. Peranan Antioksidan dalam Pembekuan Semen. *Jurnal Peternakan*. 6 (2): 63-70.

Istanty, A.S., M.A. Salim., N. Isnaini. dan T. Susilawati. 2017. Pengaruh Penggantian *Bovine Serum Albumin* (BSA) Dengan Putih Telur Dalam Pengencer Dasar CEP-2 Terhadap Kualitas Semen Kambing Boer pada Simpan Dingin. *J. Ternak Tropika*. 18(1): 1-9.

Khaira, K. 2010. Menangkal Radikal Bebas dengan Antioksidan. *Jurnal Sainstek*. 11 (2): 183-187.

Latief, M., F. Tafzi, dan A. Saputra. 2013. Aktivitas Antioksidan Ekstrak Metanol Beberapa Bagian Tanaman Kayu Manis (*Cinnamomum burmani*) Asal Kabupaten Kerinci Provinsi Jambi. Prosiding Semirata FMIPA Universitas Lampung. 233-236.

Lubis, T.M., Dasrul, C.N. Thasmi. dan Akbar. 2013. Efektivitas Penambahan Vitamin C dalam Pengencer Susu Skim Kuning Telur terhadap Kualitas Spermatozoa Kambing Boer Setelah Penyimpanan Dingin. *Jurnal Sains Pertanian*. 3 (1): 347-361.

Lukman H Y, W.Busono, S.Wahjuningsih and Suyadi. 2014. Sperm motility and viability after α-tocopherol dilution in Tris aminomethane-base extender during cold storage in bali bull. *International Journal of ChemTech Research*, 6 (14): 5726-5732.

Nurjannah, A. Azka, dan A. Abdullah. 2012. Aktivitas Antioksidan dan Komponen Bioaktif Semanggi Air (*Marsilea crenata*). *Jurnal Inovasi dan Kewirausahaan*. 1 (3): 151-158.

Pereira, G. R., E. G. Becker, L. C. Siquera, R. Ferreira,C. K. Savero, V. S. Truzzi, Oliveira and Goncalves. 2010. Assessment of Bovine Spermatozoa Viability Using Different Cooling Protocols Prior to Cryopresertion. *Italian Journal of Animal Sciene*. 9(88): 464-470.

Rochim, A., M.A. Salim, N. Isnaini, dan T. Susilawati. 2017. Pengaruh Penghilangan Rafinosa dalam Pengencer Tris Aminomethane Kuning Telur Terhadap Kualitas Semen Kambing Boer Selama Simpan Dingin. *Jurnal Ternak Tropika*. 18 (1): 27-35.

Saefudin, S. Marusin, dan Chairul. 2013. Aktivitas Antioksidan pada Enam Jenis Tumbuhan *Sterculiaceae. Jurnal Penelitian Hasil Hutan*. 31 (2): 103-109.

Sudarmanto, I., dan T. Suhartati. 2015. Aktivitas Antioksidan Senyawa Flavonoid pada Kulit Akar Tanaman Ara (*Ficus racemosa*, L). *Jurnal Kesehatan*. 6 (2): 137-141.

Susilawati, T., N. Isnaini, A.P.A. Yekti, I. Nurjannah, Errico, dan N. da Costa. 2016. Keberhasilan Inseminasi Buatan Menggunakan Semen Beku dan Semen Cair pada Sapi Pernakan Ongole. *Jurnal Ilmu-Ilmu Peternakan*. 26 (3):14-19.

Suyadi., A. Rachmawati. dan N. Iswanto. 2017. Pengaruh α-Tocopherol yang Berbeda Dalam Pengencer Dasar Tris Aminomethan Kuning Telur Terhadap Kualitas Semen Kambing Boer yang Disimpan pada Suhu 5ºC. *Jurnal Ilmu-Ilmu Peternakan*. 22(3): 1-8.

Verberckmoes, S., A. Van Soom, J. Dewulf, I. De Pauw, and A. De Kruif. 2004. Storage of fresh bovine semen in diluent based on the ionic composition cauda epididymal plasma. *J. Reprod.Dom. Anim*. 39(6):1-7.

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