

# THE ROLE OF GUAVA FRUIT FILTRATE ON MAINTAINING THE PRE-FREEZING AND POST- THAWED QUALITY OF BALI BULL SPERMATOZOA

*by I Wayan Lanus Sumadisa Sumadisa*

---

**Submission date:** 22-Feb-2023 06:24PM (UTC-0600)

**Submission ID:** 2020824424

**File name:** THE\_ROLE\_OF\_GUAVA\_FRUIT\_FILTRATE\_ON\_MAINTAINING\_TH.pdf (104.85K)

**Word count:** 3854

**Character count:** 20699

## THE ROLE OF GUAVA FRUIT FILTRATE ON MAINTAINING THE PRE-FREEZING AND POST-THAWED QUALITY OF BALI BULL SPERMATOZOA

I Wayan Lanus Sumadiasa<sup>1\*</sup>, Lukman<sup>1</sup>, and Rodiah<sup>1</sup>

<sup>1</sup>Animal Reproduction Laboratory, Faculty of Animal Husbandry, Mataram University, Nusa Tenggara Barat, Indonesia

\*Corresponding author: wlanuss@gmail.com

### ABSTRACT

This study aimed to determine the role of guava fruit filtrate (GFF) on maintaining the pre-freezing and post-thawed quality of Bali bull spermatozoa in tris-egg yolk (TEY) extender. The bovine semen was collected using artificial vagina twice a week (n= 10). Ejaculate samples were divided into four tubes and each tube was respectively added the following diluents: TEY, TEY + 5% GFF, TEY + 10% GFF, and TEY + 15% GFF. The diluted semen was filled into the straws (0.25 mL) and cooled in refrigerator at 4° C for 2.5 hours. The samples were kept on vapor phase of liquid nitrogen (N<sub>2</sub>) (-120° C) for 10 minutes prior to be stored in liquid phase (-196° C). The quality of pre-freezing and post-thawed spermatozoa in straw sample (i.e. motility, viability, and abnormality) was evaluated under light microscopy at 400x magnification. Data were analysed statistically. The percentage of motility and viability both on pre-freezing and post-thawed process were significantly higher in semen diluted with TEY + 10% GFF compared to control (TEY), TEY + 5% GFF, and TEY + 15% GFF. Addition of 10% GFF into tris-egg yolk extender play role for maintaining the quality of pre-freezing and post-thawed Bali bull spermatozoa.

Key words: bovine, guava filtrate, pre-freezing and post-thawed, spermatozoa

### ABSTRAK

Penelitian ini bertujuan mengetahui peran filtrat buah jambu biji (FBJB) dalam mempertahankan kualitas spermatozoa sapi Bali sebelum dan setelah pembekuan di dalam pengencer tris-kuning telur (TKT). Semen sapi ditampung dengan vagina buatan dua kali sehari (n= 10). Sampel semen dibagi dalam empat tabung reaksi, kemudian masing-masing ditambahkan pengencer TKT, TKT + 5% FBJB, TKT + 10% FBJB, dan TKT + 15% FBJB. Semen yang telah diencerkan dimasukkan ke dalam straw (0,25 ml), kemudian didinginkan di dalam kulkas suhu 4° C selama 2,5 jam. Selanjutnya dilakukan prapembekuan di atas uap nitrogen (N<sub>2</sub>) cair suhu -120° C selama 10 menit. Pembekuan dilakukan dengan cara dicelupkan ke dalam N<sub>2</sub> cair suhu -196° C dan disimpan di dalam kontainer berisi N<sub>2</sub> cair. Kualitas spermatozoa pada sampel prapembekuan dan setelah thawing dievaluasi di bawah mikroskop cahaya pembesaran 400x meliputi motilitas, viabilitas, dan abnormalitasnya. Data dianalisis secara statistik. Hasil penelitian adalah persentase motilitas dan viabilitas spermatozoa tertinggi dan abnormalitas terendah terdapat pada pengencer TKT + 10% FBJB jika dibandingkan dengan perlakuan kontrol (TKT), 5% FBJB dan 15% FBJB, baik pada proses prapembekuan maupun setelah thawing. Penambahan 10% FBJB di dalam pengencer tris-kuning telur berperan dalam mempertahankan kualitas spermatozoa sapi Bali sebelum dan setelah pembekuan.

Kata kunci: sapi, filtrat jambu biji, sebelum dan setelah pembekuan, spermatozoa

### INTRODUCTION

Washing, diluting, cooling, freezing, and thawing can change the surface protein of spermatozoa, causing instability and decreasing fertility (Gillan *et al.*, 2004; Leahy and de Graaf, 2012). Lipid peroxidation during cryopreservation can increase the sensitivity of spermatozoa toward reactive oxygen species (ROS) and reduce motility and viability up to 50% (Vishwanath and Shannon, 2000; Andrabi *et al.*, 2008). Spermatozoa mortality rate reached more than 50% during frozen sperm making and thawing process (Delgado *et al.*, 2009; Kaeoket *et al.*, 2011).

Death and damage of spermatozoa during handling produce ROS including hydroxyl radical, anion superoxide radical, hydrogen peroxide, singlet oxygen, hypochlorite, nitrate oxide and peroxytrite radical. Free radicals play role as oxidant or reductant and damage DNA, protein, carbohydrate, lipid, acid, and nuclear protein by delivering or receiving electron from other molecules in cell nucleus or cell membrane (Lobo *et al.*, 2010).

Seminal plasma has antioxidant system for protection against ROS activity. However addition of diluents to the semen can reduce antioxidant level and ruin the balance of ROS and endogenous antioxidants

leading to spermatozoa damage (Rao and Rao, 2007; Kothari *et al.*, 2010). Antioxidants supplementation in diluents is needed to protect spermatozoa (Asadpour *et al.*, 2011; Bansal and Bilaspuri, 2011), reduce damage caused by free radical and ROS, as well as to protect sperm vitality (Tavilani *et al.*, 2008; Stevanov *et al.*, 2011).

Antioxidant can be produced both endogenously by the body and exogenously from daily foods and beverage (Ibrahim *et al.*, 2008). There are two types of antioxidants, specifically enzymatic and non-enzymatic. Enzymatic (superoxide dismutase, catalase, glutathione, and alpha lipoic acids) and non-enzymatic antioxidants (vitamin E, C, and A) are generally efficient against free radical. Vitamin E and C protects against free radicals by maintaining structural and functional integrity of the spermatozoa. Vitamin C is a hydrophilic vitamin which cannot be stored endogenously and must be consumed or supplemented. Low concentration of vitamin C (ascorbic acid) acts as oxidant, while high concentration (±5 mM) acts as antioxidant (Andrabi *et al.*, 2008).

Adding antioxidant to the semen diluents is necessary to prevent cell damage by breaking the oxidation reactions chain and protect molecules from oxidation activity by oxidizing their own molecules

(Norshazila *et al.*, 2010; Bansal and Bilaspuri, 2011). Antioxidant in diluents can act as electron receiver (Funahashi and Sano, 2005). Antioxidant molecules reduce oxidative stress effect, while internal compound protect cells from oxygen radicals that can increase the spermatozoa quality (Kulaksiz and Daskin, 2010).

Currently, there are a lot of attentions about the use of natural antioxidant to inhibit lipid peroxide and prevent sperm cell damage (Hui-Yin and Gow-Chin, 2007). This new innovative diluents are expected to preserve and protect the quality of spermatozoa, further improving its fertility capability.

Guava fruit (*Psidium guajava* Linn) contains many antioxidants including polyphenol, vitamin C, A, B, and E, and carotenoid. It was found a good result when added guava fruit filtrate (GFF) to the CEP-2 diluents for cow spermatozoa preservation at 5° C (Sumadiasa *et al.*, 2015). Addition of GFF in tris-egg yolk (TEY) to maintain the quality of Bali bull pre-freezing and post-thawing spermatozoa needs to be evaluated to increase semen post-thawing quality. The combination of TEY and GFF is expected as source of nutrition, energy, macro-protectant, and antioxidant.

## 22 MATERIALS AND METHODS

1 Semen samples were collected from 4 cows at Regional Artificial Insemination that belongs to Livestock and Animal Health Office West Nusa Tenggara Province which were taken in rotation using artificial vagina, 2 times a week for 5 weeks (n= 10). The ejaculated semen was immediately examined macroscopically and microscopically to determination of its quality. The criteria for semen used in this study were having spermatozoa with >70% motility and viability, less than 20% abnormality, and had concentration of ± 1000 million/mL. This was a randomized-group experimental study which involved 4 groups of treatment i.e. P<sub>0</sub>, P<sub>1</sub>, P<sub>2</sub>, and P<sub>3</sub>.

### Guava Fruit Filtrate (GFF) Production

Guavas were blended and dissolved in double distilled water with 1:2 ratios. The solution was then centrifuged at 3500 rpm for 10 minutes. The supernatant was infiltrated using millipore membrane (Sartorius stedim Minisart®) 0.42 µm continue with 0.2 µm to obtain the filtrate. The filtrate was pasteurized in boiling water at 60° C for 3 minutes, then put in microtube 2.5 mL and stored in refrigerator at 5° C (Sumadiasa *et al.*, 2015).

### Tris-Egg Yolk (TEY) Production

Tris buffer contained 3.634 g tris (hydroxymethyl) aminomethane, 0.5 g glucose, and 1.99 g citric acid, and was boiled at 90° C in 100 mL double distilled water for 10 minutes and then left out until 32° C. After that, the buffer was added by 0.1 g streptomycin and 0.06 g penicillin, then homogenized. Finally, the buffer added by 10 mL egg yolk and 10% glycerol. Buffer solution was put in reaction tube P<sub>0</sub> (TEY only), P<sub>1</sub> (TEY + 5% GFF), P<sub>2</sub> (TEY + 10% GFF), and P<sub>3</sub> (TEY

+ 15% GFF) (modification from Toelihere, 1993; Sumadiasa, 2015).

### Dilution, Straw Filling, and Equilibration

Stored semen were diluted by each media with concentration of spermatozoa as much as 80 million/mL and then filled in straw (0.25 mL) so that every straw contain at least 20 million spermatozoa. The filled straw from room temperature was equilibrated in refrigerator to reach 5° C for ±2.5 hours. After that, pre-freezing method was conducted by putting the straw on liquid nitrogen (N<sub>2</sub>) vapor for 10 minutes and then dipped into liquid N<sub>2</sub> and stored as frozen semen.

### The Evaluation of Spermatozoa Quality Before and After Freezing Treatment

The preservation and protection ability of GFF towards spermatozoa quality was evaluated. After pre-freezing period, straw samples were warmed up in water bath at 42° C and then evaluated (motility, viability, and abnormality) under microscope with 10x40 magnifications. The quality evaluation of post-thawing spermatozoa was performed on frozen-straw semen after being stored for 15-30 minutes and warmed up (thawing) in water bath at 42° C.

### Data Analysis

10 Data was analysed using analysis of variance (ANOVA) continued with Duncan Multiple Range Test (DMRT).

## RESULTS AND DISCUSSION

1 The bulls at Regional Artificial Insemination that belongs to Livestock and Animal Health Office West Nusa Tenggara (NTB) Province were intensively maintained for more than 3-5 years and were a semen producer to supply all districts in NTB. The evaluation toward semen quality showed that this study used a good and worth processing semen.

The mean semen volume from 10 times collection were 7.5±2.35 mL and similar to semen volume found in exotic cows ranged from 5-8 mL (Garner and Hafez, 2008); 4.84±0.01 mL (Patel and Siddiquee, 2013); 10.5 mL (Hossain *et al.*, 2012). The color and consistency of semen were in normal range with pH 6.2 which was between normal range of the bull semen ranged from 5.15-6.53 (Rehman *et al.*, 2012), 6.2-6.4 (Yavas and Daskin, 2012), and 6.4-6.88 (Patel and Siddiquee, 2013). The condition was presumably due to differences in bull types, temperature or season when the semen was collected, or the type of feed, whereas the weather at the study was quite good and normal rain intensity.

Spermatozoa mass motility was ++, progressive motility was 70±0%, the number of lives spermatozoa was quite high at 79.8±4.39%, and the abnormality was very low at 8.6±3.06%. Macroscopic examination showed spermatozoa concentration more than 1000 million cell/mL (121.36±32.88 x 10<sup>7</sup>), which was higher than reported by Andrabi *et al.* (2002), Hossain

et al. (2012), and Abavisani et al. (2013) i.e.  $0.81 \times 10^9$ /mL and was close to the average concentration of  $1.32 \times 10^9$ /mL (Sarder, 2007) and  $1.25 \times 10^9$  (Patel and Siddiquee, 2013).

We found a good average of pre-freezing spermatozoa that was feasible to be frozen. Table 1 showed the average value of motility, viability, and abnormality of pre-freezing Bali bull spermatozoa dissolved in TEY + GFF. The average quality of spermatozoa dissolved in 10% GFF + TEY was quite good and there were different quality in every treatment. Addition of 10% GFF to the TEY gave highest mean percentage of motility and viability as well as lowest abnormality of spermatozoa. The motility of spermatozoa would be very low by adding 15% GFF into TEY. In addition, control group (TEY only) showed lowest viability and highest abnormality of spermatozoa.

The mean motility of spermatozoa in group P2 was statistically higher than group P3 ( $P < 0.01$ ), but was not statistically different with group P0 and P1. Generally, the best treatment for maintaining the pre-freezing Bali bull sperm was by adding 10% of GFF into TEY. It was expected that the combination of tris, egg yolk, and guava fruit filtrate could be synergized for preserving and protecting the spermatozoa during pre-freezing process.

The quality of spermatozoa can be maintained everlastingly after being frozen and stored in liquid  $N_2$  at  $-196^\circ C$  as long as the liquid  $N_2$  was still available in the containers. It mean that there was no quality difference between 15 minutes and 1 year or even several years of storage, unless the amount of liquid  $N_2$  was reduced which cause decreasing storage temperature of lower than  $-196^\circ C$ . After being thawed, the spermatozoa still showed a high mean percentage of motility and viability above the average quality of sperm that is suitable for artificial insemination specifically 50% of minimum motility and viability and 20% maximum of abnormality.

Table 2 provided the average value of motility, viability, and abnormality of post-thawing Bali bull spermatozoa in all groups. In post thawing condition, spermatozoa dissolved in TEY + 10% GFF had highest mean percentage of motility and viability as well as lowest mean percentage of abnormality, whereas addition of 15% GFF to the TEY provided lowest mean percentage of motility and viability. In addition, highest

abnormality of spermatozoa was found in control group. The mean motility of post-thawing spermatozoa in group P2 was statistically higher than all groups ( $P < 0.01$ ), but the viability and abnormality were not statistically different.

The best quality of spermatozoa in both pre-freezing and post-thawing was found in group P2 by adding 10% GFF into TEY. The P2 diluents had good combination because GFF provide antioxidant properties to protect spermatozoa from ROS damage, while egg yolk provides nutrition, energy, and low density lipoprotein (LDL) as membrane cell protector.

The abnormality of spermatozoa begins as membrane damaged by ROS. The ROS has an ability in adhere at the ovum to ruin fertilization process and damage the DNA material inside spermatozoa. LDL in egg yolk could reduce the membrane phospholipid loss, stabilized membrane, and seized toxins from seminal plasma proteins (Akhter et al., 2010).

Phospholipid (lecithin) and LDL contained in egg yolk prevent the cold shock phenomenon. Both of the components build an interfacial cell layer during freezing or equilibration process thus causing an increase in phospholipid and cholesterol bonds to the plasma membrane of spermatozoa. Further, it builds up a complex with seminal protein plasma which make the lipid cannot work inside the membrane cell (Chanapiwat et al., 2011; Trout, 2012).

Antioxidant is an active material which is very important to protect spermatozoa during storage. Antioxidant system such as glutathione (GSH), GPX, catalase (CAT) and SOD can neutralize ROS. Moreover, addition of several non-enzymatic molecule including glutathione, thioredoxin, vitamin D, E, and C in semen work against lipid peroxide, maintain the motility and viability of spermatozoa (Zeitoun and Al-Damegh, 2015).

Guava fruit (*Psidium guajava* Linn) contains several antioxidant and important component to protect the cell i.e. carotene, vitamin B1 and B2, niacin, fiber, vitamin C, lutein, lycopene, and zeaxanthine (Rahmat et al., 2006). Vitamin C content in guava was 50-300 mg/100 g fresh weight (Thaipong et al., 2005), even reaching 350-450 mg before ripe (Rao and Rao, 2007). Therefore, GFF could significantly maintain the quality of spermatozoa pre-freezing and post-thawing process.

**Table 1.** The average of pre-freezing spermatozoa quality (n= 10)

Variable	Treatment			
	P0	P1	P2	P3
Motility	63.0±4.4 <sup>b</sup>	60.3±5.5 <sup>b</sup>	65.8±6.1 <sup>b</sup>	52.8±7.0 <sup>a</sup>
Viability	79.2±3.1 <sup>a</sup>	81.0±2.7 <sup>a</sup>	87.4±3.5 <sup>b</sup>	80.9±3.8 <sup>a</sup>
Abnormality	21.1±4.3 <sup>b</sup>	20.0±3.6 <sup>ab</sup>	17.4±2.8 <sup>a</sup>	20.4±3.5 <sup>ab</sup>

<sup>a,b</sup>Different superscripts in the same row indicate significant different ( $P < 0.01$ )

**Table 2.** The average of post-thawing spermatozoa quality (n= 10)

Variabel	Treatment			
	P0	P1	P2	P3
Motility	52.8±4.2 <sup>a</sup>	51.0±5.9 <sup>a</sup>	61.0±3.2 <sup>b</sup>	49.3±7.6 <sup>a</sup>
Viability	76.8±4.8 <sup>ab</sup>	76.7±6.1 <sup>ab</sup>	81.4±4.7 <sup>b</sup>	76.1±4.9 <sup>a</sup>
Abnormality	23.1±3.0 <sup>b</sup>	22.4±3.1 <sup>b</sup>	19.5±3.3 <sup>a</sup>	22.6±2.8 <sup>b</sup>

<sup>a,b</sup>Different superscripts in the same row indicate significant different ( $P < 0.01$ )

Vitamin C content in GFF could neutralize toxins in semen, suppress the rate of cholesterol catabolism, and reduce spermatozoa attachment to the cluster and make them move and swim easily. High antioxidant intake from vitamin C, E, and  $\beta$ -carotene could significantly maintain the motility of spermatozoa. Supplementation of 5 mM vitamin C or vitamin E into diluents could decrease the percentage of spermatozoa abnormality after storage (Mittal *et al.*, 2014).

## CONCLUSION

Guava fruit filtrate (GFF) in tris-egg yolk (TEY) dilution has important role in maintaining the quality of Bali bull spermatozoa before and after frozen. The addition of 10% GFF into TEY is very effective in preserve and protect the spermatozoa quality both before and after frozen.

## ACKNOWLEDGEMENT

The research team expressed big gratitude to the directorate of Research and Community Service for funding the research implementation. The research team stated that there was no conflict of interest from other parties during writing the study results.

## REFERENCES

- Abavisani, A., J. Arshami, A.A. Naserian, M.A.S. Kandelousi, and M. Azzadeh. 2013. Quality of bovine chilled or frozen-thawed semen after addition of omega-3 fatty acids supplementation to extender. *Int. J. Fertil. Steril.* 7(3):161-168.
- Akhter, S., B.A. Rakha, S.M.H. Andrabi, and M.S. Ansari. 2010. Comparison of egg yolks from three avian species in extender for cryopreservation of Sahiwal bull epididymal spermatozoa. *Anim. Sci. Pap. Rep.* 29(2):131-138.
- Andrabi, S.M.H., S. Naheed, L.A. Khan, and N. Ullah. 2002. Semen characteristics of crossbreed (Friesian x Sahiwal) bulls at livestock research station, National Agricultural Research Centre, Islamabad. *Pakistan Vet. J.* 22(4):181-187.
- Andrabi, S. M.H., M.S. Ansari, N. Ullah, and M. Afzal. 2008. Effect of non-enzymatic antioxidants in extender on post-thaw quality of buffalo (*Bubalus bubalis*) bull spermatozoa. *Pakistan Vet. J.* 28(4):159-162.
- Asadpour, R., R. Jafari, and H. Tayefi-Nasrabad. 2011. Influence of added vitamin C and vitamin E on frozen-thawed bovine sperm cryopreserved in citrate and tris-base extenders. *Vet. Res. Forum.* 2(1):37-44.
- Bansal, A.K. and G.S. Bilaspuri. 2011. Impacts of oxidative stress and antioxidants on sperm functions. *Vet. Med. Int.* <http://dx.doi.org/10.4061/2011/686137>.
- Chanapiwat, P., K. Kaeoket, and P. Tummaruk. 2011. Cryopreservation of boar sperm by egg yolk-based extenders containing lactose or fructose is better than sorbitol. *J. Vet. Med. Sci.* 74(3):351-354.
- Delgado, P.A., T.D. Lester, and R.W. Rorie. 2009. Effect of low-sodium, choline-based diluents on viability of bovine sperm stored at refrigerator temperatures. *AAES Research Series.* 574:77-79.
- Funahashi, H. and T. Sano. 2005. Collected antioxidants improve the function of extended boar sperm stored at 10° C. *Theriogenology.* 63(6):1605-1616.
- Garner, D.L. and E.S.E. Hafez. 2008. Spermatozoa and Seminal Plasma. In *Reproduction in Farm Animals*. Hafez, B. and E.S.E. Hafez (Eds.). 7<sup>th</sup> ed. Blackwell Publishing, New Jersey.
- Gillan, L., W.M.C. Maxwell, and G. Evans. 2004. Preservation and evaluation of sperm for artificial insemination. *Reprod. Fertil. Dev.* 16(4):4470-452.
- Hossain, M.E., M.M. Khatun, M.M. Islam, and O.F. Miazi. 2012. Semen characteristics of breeding bulls at the central cattle breeding and dairy farm of Bangladesh. *Bang. J. Anim. Sci.* 41(1):1-5.
- Hui-Yin, C. and Y. Gow-Chin. 2007. Antioxidant activity and free radical-scavenging capacity of extracts from guava (*Psidium guajava* L.) leaves. *J. Food Chem.* 101:686-694.
- Ibrahim, S.F., K. Osman, S. Das, A.M. Othman, N.A. Majid, and M.P.Ab. Rahman. 2008. A study of the antioxidant effect of alpha lipoic acids on sperm quality. *Clinics.* 63(4):545-550.
- Kaeoket, K., P. Chanapai, P. Junchiyaphoom, and P. Chanapiwat. 2011. The effect of using long term and short term extenders during cooling process on the quality of frozen boar sperma. *Thai J. Vet. Med.* 41(3):283-288.
- Kothari, S., A. Thomson, A. Agarwal, and S.S. du Plessis. 2010. Free radical: Their beneficial and detrimental effects on sperm function. *Indian J. Exp. Biol.* (48):425-435.
- Kulaksiz, R. and A. Daskin. 2010. In vitro evaluation of Saanen buck sperm frozen in different extenders supplemented with various antioxidants. *Ankara Üniv. Vet. Fak. Derg.* (57):151-156.
- Leahy, T. and S.P. de Graaf. 2012. Seminal plasma and its effect on ruminant spermatozoa during processing. *Reprod. Dom. Anim.* 47(4):207-213.
- Lobo, V., A. Patil, A. Phatak, and N. Chandra. 2010. Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacogn. Rev.* 4(8):118-126.
- Mittal, P., Vikas Gupta, Gurpreet Kaur, Ashish K. Garg, and Amarjeet Singh. 2010. Phytochemistry and pharmacological activities of *Psidium guajava*. *IJPSR.* 1(9):9-19.
- Norshazila, S., S.I. Zahir, K.M. Suleiman, M.R. Aisyah, and K.K. Rahim. 2010. Antioxidant levels and activities of collected seeds of Malaysian tropical fruits. *Mal. J. Nutr.* 16(1):149-159.
- Patel, B.R. and G.M. Siddiquee. 2013. Physical and morphological characteristics of Kankrej bull semen. *Vet. World.* 6(7):405-408.
- Rahmat, A., M.F.A. Bakar, and Z. Hambali. 2006. The effects of guava (*Psidium guajava*) consumption on total antioxidant and lipid profile in normal male youth. *AJFAND.* 6(2):1-12.
- Rao, A.V. and L.G. Rao. 2007. Carotenoids and human health. *Pharmacol. Res.* 55:207-216.
- Rehman, Z.U., M.U. Samo, T.A. Qureshi, S. Khan, M.S. Qureshi, F.A. Khan, and S. Bahadadr. 2012. Studies on the freezability of Kundhi buffalo bull semen. *J. Anim. Plant Sci.* 22(2):18-23.
- Sarder, M.J.U. 2007. Environment related variations in the semen characteristics of bulls used for artificial insemination (AI) programme in Bangladesh. *Univ. J. Zool.* 26:81-88.
- Stevanov, R., M. Sabev, and M. Ivanova-Kicheva. 2011. Protective effect of the antioxidant enzyme on sperm from Bubalus bubalis type after cryoconservation. *Biotechnol. Anim. Husbandry.* 27(3):934-949.
- Sumadiasa, I.W.L. 2015. Substitusi Kuning Telur di Dalam Pengencer CEP-2 terhadap Kualitas dan Fertilitas Spermatozoa Sapi Bali Selama Simpan Dingin. *Disertasi*. Universitas Brawijaya. Malang.
- Sumadiasa, I.W.L., T. Susilawati, G. Ciptadi, and N. Isnaini. 2015. The potency of guava filtrate (*Psidium guajava* Linn) for preservation of Bali bull spermatozoa. *IOSR-JAVS.* 8(5):51-57.
- Tavilani, H., M.T. Goodarzi, A. Vaisi-raygani, S. Salimi, and T. Hassanzadeh. 2008. Activity of antioxidant enzymes in seminal plasma and their relationship with lipid peroxidation of spermatozoa. *Int. Braz. J. Urol.* 34(4):485-491.
- Thaipong, K., U. Boonprakob, L. Cisneros-Zevallos, and D. H. Bime. 2005. Hydrophilic and lipophilic antioxidant activities of guava fruits. *Shoutheast Asian J. Trop. Med. Pub. Health.* 36(4):254-257.
- Toelhiere, M.R. 1993. *Inseminasi Buatan pada Ternak*. Penerbit Angkasa, Bandung.
- Trout, S.W. 2012. Evaluation of Different Concentrations of Egg Yolk in Canine Frozen Semen Extender. *Thesis*. Biomedical and Veterinary Sciences, Faculty of the Virginia Polytechnic, Institute and State University, Virginia.
- Vishwanath, R. and P. Shannon. 2000. Storage of bovine sperm in liquid and frozen state. *Anim. Reprod. Sci.* 62:23-33.
- Yavas, T.K. and A. Daskin. 2012. Effect of alternative cryopreservation procedures on bull sperm. *Ankara Üniv. Vet. Fak. Derg.* 59:231-234.
- Zeitoun, M.M. and M.A. Al-Damegh. 2015. Effect of nonenzymatic antioxidants on sperm motility and survival relative to free radicals and antioxidant enzymes of chilled-stored ram semen. *Open J. Anim. Sci.* (5):50-58.

# THE ROLE OF GUAVA FRUIT FILTRATE ON MAINTAINING THE PRE-FREEZING AND POST-THAWED QUALITY OF BALI BULL SPERMATOZOA

## ORIGINALITY REPORT

13%

SIMILARITY INDEX

10%

INTERNET SOURCES

5%

PUBLICATIONS

3%

STUDENT PAPERS

## PRIMARY SOURCES

1	<a href="https://es.scribd.com">es.scribd.com</a> Internet Source	3%
2	<a href="https://krishikosh.egranth.ac.in">krishikosh.egranth.ac.in</a> Internet Source	1%
3	<a href="https://repository.ub.ac.id">repository.ub.ac.id</a> Internet Source	1%
4	<a href="https://jurnalgreenswarnadwipa.blogspot.com">jurnalgreenswarnadwipa.blogspot.com</a> Internet Source	1%
5	Submitted to Pascasarjana Universitas Negeri Malang Student Paper	1%
6	Adel Saberivand, Seddigheh Pashapour, Amirhessam Eskafi Noghani, Zahra Namvar. "Synergistic effect of Royal jelly in combination with glycerol and dimethyl sulfoxide on cryoprotection of Romanov ram sperm", Cryobiology, 2021 Publication	<1%

7	<a href="http://eprints.unram.ac.id">eprints.unram.ac.id</a> Internet Source	<1 %
8	<a href="http://hau.repository.guildhe.ac.uk">hau.repository.guildhe.ac.uk</a> Internet Source	<1 %
9	<a href="http://ojs.uho.ac.id">ojs.uho.ac.id</a> Internet Source	<1 %
10	<a href="http://www.sysrevpharm.org">www.sysrevpharm.org</a> Internet Source	<1 %
11	Ejaz Ahmad, Zahid Naseer, Melih Aksoy. "Glutathione supplementation in semen extender improves rabbit sperm attributes during refrigeration", World Rabbit Science, 2021 Publication	<1 %
12	<a href="http://link.springer.com">link.springer.com</a> Internet Source	<1 %
13	<a href="http://researcherslinks.com">researcherslinks.com</a> Internet Source	<1 %
14	<a href="http://aff.fkh.ipb.ac.id">aff.fkh.ipb.ac.id</a> Internet Source	<1 %
15	<a href="http://amb-express.springeropen.com">amb-express.springeropen.com</a> Internet Source	<1 %
16	<a href="http://www.scielo.br">www.scielo.br</a> Internet Source	<1 %

17

Alcay, Selim, Elif Gokce, M. Berk Toker, N. Tekin Onder, Burcu Ustuner, Ender Uzabacı, Zulfiye Gul, and Seda Cavus. "Freeze-dried egg yolk based extenders containing various antioxidants improve post-thawing quality and incubation resilience of goat spermatozoa", *Cryobiology*, 2016.

Publication

&lt;1 %

18

[www.e-jarb.org](http://www.e-jarb.org)

Internet Source

&lt;1 %

19

Faridullah Hashim, Eva Tvrdá, Peter Massányi, Robert Stawarz, Norbert Lukáč. "Effects of biological active substances to the spermatozoa quality", *Journal of Microbiology, Biotechnology and Food Sciences*, 2016

Publication

&lt;1 %

20

Napa Siwarungson, Imran Ali, Thanakorn Damsud. "Comparative analysis of antioxidant and antimelanogenesis properties of three local guava (*Psidium guajava* L.) varieties of Thailand, via different extraction solvents", *Journal of Food Measurement and Characterization*, 2013

Publication

&lt;1 %

21

Pinter, J.. "Induction of Ovulation of Mature Oocytes by the Maturation-Inducing Steroid 17,20@b,21-Trihydroxy-4-pregnen-3-one in

&lt;1 %



the Spotted Seatrout", General and Comparative Endocrinology, 199908

Publication

22

[psasir.upm.edu.my](http://psasir.upm.edu.my)

Internet Source

<1 %

23

[vetjournal.ankara.edu.tr](http://vetjournal.ankara.edu.tr)

Internet Source

<1 %

24

[yudihermawan12.blogspot.com](http://yudihermawan12.blogspot.com)

Internet Source

<1 %

25

A P A Yekti, W D Cahyo, S Wahjuningsih, Kuswati, A N Huda, T Susilawati. "The Decreasing of Quality Liquid Semen Using Four Months Storages of Tris Aminomethan and CEP-3 Diluents", IOP Conference Series: Earth and Environmental Science, 2020

Publication

<1 %

26

S. A. H. Shah, S. M. H. Andrabi, I. Z. Qureshi. "Freezability of water buffalo bull ( ) spermatozoa is improved with the addition of curcumin (diferuoyl methane) in semen extender ", Andrologia, 2017

Publication

<1 %

Exclude quotes Off

Exclude matches Off

Exclude bibliography On

# THE ROLE OF GUAVA FRUIT FILTRATE ON MAINTAINING THE PRE-FREEZING AND POST-THAWED QUALITY OF BALI BULL SPERMATOZOA

---

GRADEMARK REPORT

---

FINAL GRADE

**/0**

GENERAL COMMENTS

**Instructor**

---

PAGE 1

---

PAGE 2

---

PAGE 3

---

PAGE 4

---