Red Watermelon Fruit Extract (Citrullus Lanatus) In Egg Yolk Tris Diluent In Maintaining The Quality Of Goat Peanut Spermatozoa At 5°C

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Submission date: 15-Feb-2023 09:11PM (UTC-0600)
Submission ID: 2015336469
File name: 4856-11750-1-PB_1.pdf (233.44K)
Word count: 4151
Character count: 21298

International Journal of Progressive Sciences and Technologies (IJPSAT)
ISSN: 2509-0119.

Vol. 36 No. 1 December 2022, pp. 454-460

Red Watermelon Fruit Extract (Citrullus Lanatus) In Egg Yolk Tris Diluent In Maintaining The Quality Of Goat Peanut Spermatozoa At 5°C

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Abstract – This study aims to determine the effect of adding watermelon fruit extract to egg yolk tris diluent to maintain the quality of spermatozoa of kacang goat. Knowing the best concentration of watermelon extract in maintaining the sperm quality of kacang goat stored at 50C. This study used a completely randomized design (CRD) with four treatments and four replications. The material used in this study was semen from 2-year-old kacang goats. Semen is collected using an artificial vagina twice a week. Watermelon extract concentrations were P0, P1, P2 and P3 (0%, 1.5%, 2.5% and 3.5%). The variables observed were progressive motility, viability and abnormality. The data obtained were analyzed using analysis of variance (ANAVA), significantly different results were further tested with Duncan's test in the SPSS16 program. The results showed a significantly better effect (P>0.05) on progressive motility, viability and abnormalities. Progressive motility at P0 32±2.58%, P1 40±6.32%, P2 50±2.68%, and P3 35±4.47%. Spermatozoa viability for P0 was 58±4.96%, P1 was 67.2±3.19%, P2 was 68.3±3.27%, and P3 was 69.2±1.17%. Spermatozoa abnormalities for P0 13.8 ± 0.75%, P1 11.3 ± 0.82% , P2 6.8 ± 1.17 % and P3 12.3 ± 0.82 %. The best concentration obtained by adding watermelon fruit extract was 2.5% (P2) with progressive motility of 50 ± 2.68%, viability of 68.3 ± 3.27%, and abnormality of 6.3 ± 0.82% during four days of storage. .

Keywords – red watermelon extract, kacang goat, spermatozoa, egg yolk tris, temperature 5°c

I. INTRODUCTION

Peanut goat is one of the local goat breeds which is very widely distributed throughout Indonesia. Judging from the level of productivity and reproducibility which varies greatly in each region. This type of goat nation in total population decreases every year. It is feared that one day this kacang goat will become extinct if excavation and preservation are not carried out again.

One of the efforts that can be made to increase the livestock population is to apply artificial insemination (AI) reproductive technology by means of processing semen which consists of storing, diluting and storing semen. The elements that can be used in the manufacture of cement diluent consist of several chemicals that function as a source of energy, buffer, protector of spermatozoa and antibiotics as controlling elements for the development of microorganisms.

One of the ingredients that play a role in diluting semen is egg yolk tris which functions as a buffer, stabilizes pH, maintains electrolytes and protects spermatozoa from cold chok), which is a solution containing fructose and citric acid (Hoesni, 1997). Tris yolk is a good buffer solution with good osmotic pressure, electrolytes and pH balance (Affandhy et al., 2003).

The diluents are not toxic to spermatozoa, are isotonic, contain elements whose physical and chemical properties are almost the same as semen, can still maintain spermatozoa fertility, contain buffers, contain energy sources and inhibit bacterial growth (Susilawati, 2011).

Tris diluent containing egg yolk is urgently needed, because egg yolk contains lipoprotein and lecithin which can reduce the effect of cold shock on spermatozoa, so that damage during dilution, cooling by freezing will be reduced (Salisbury and Van Demark, 1985).

Egg yolk contains glucose which functions as an energy source for spermatozoa. Egg yolk also has protein, water-soluble vitamins, oil and has a viscosity that contains spermatozoa. In addition, egg yolk is able to maintain the motility and integrity of the acrosome, the mitochondrial membrane of spermatozoa and also the osmotic buffer properties, so that spermatozoa cells are more tolerant of hypertonic diluents (Jones and Martin, 1973).

Red watermelon is a fruit that is popular with Indonesian people because it is easy to get and has a sweet taste. The meat section contains lots of carbohydrates, vitamins and protein. In addition, watermelon contains vitamin C, beta-carotene and lycopene (Johnson et al., 2013). Previous studies have shown that vitamin C found in watermelon, beta-carotene and lycopene in diluent can increase the quality of the same spermatozoa during storage (Rizal, 2005; Savitri et al., 2014; Siahaan et al., 2012).

Based on the description above, researchers are interested in conducting research on the addition of watermelon extract in egg yolk tris dilution on the quality of spermatozoa of kacang goat at 5^0C.

II. Method

The material used in this study was semen from 2-year-old kacang goats. Semen was collected using an artificial vagina twice a week with the criteria for mass motility ≥ 2+, individual motility ≥ 75%.

- **Diluent Material**

The materials used in this study were red watermelon, chicken egg yolk, 1.99 g citric acid, 0.5 g fructose, 0.06 g penicillin, 0.1 g. Using a completely randomized design (CRD) with four treatments, namely P0 = 9.75 ml tris + 2.5% egg yolk, P1 = 9.65 ml tris + 2.5% egg yolk + 1% Red Watermelon Extract (ESM), P2 = 9.55 ml tris + 2.5% egg yolk + 2% ESM and P3 = 9.45 ml tris + 2.5% egg yolk + 3% ESM and each treatment was repeated six times.

- **Research Materials**

The material used in this study was semen from 2-year-old kacang goats. Semen is collected using an artificial vagina twice a week. Macroscopic assessment of spermatozoa includes volume, color, consistency (thickness) and pH, while microscopically assesses mass movement and individual spermatozoa.

The research method used was laboratory research using a completely randomized design (CRD). This study was to determine the quality of semen spermatozoa of Kacang goats at 5^0C storage.

- **Data analysis**

The data obtained were analyzed using analysis of variance (ANAVA) based on a completely randomized design (CRD). If the analysis results are significantly different (P <0.05), then the Duncan's test will be continued in the SPSS 16 program.

III. Results And Discussion

Motility is one of the determining criteria for determining semen quality as seen from the number of progressively motile spermatozoa compared to all existing spermatozoa in one microscope view. Progressive motility is one of the absolute parameters of the quality of semen to be processed for artificial insemination purposes. The results of observing the progressive motility of spermatozoa of kacang goat after dilution and addition of watermelon fruit extract at 5^0C for 4 days can be seen in Table 3 below.

Table 1. Percentage of progressive motility of kacang goat spermatozoa in watermelon extract at 5^0C storage for 5 days.

Storage time (Days)	Treatment of watermelon fruit extract concentration (%)			
	0	1	2	3
0	53±2,74^{a}	60±4,47^{b}	72±1,94^{c}	61±3,76^{b}
1	47±2,58^{a}	55±4,47^{bc}	67±2,53^{c}	56±3,76^{b}
2	43±2,74^{a}	49±4,92^{b}	62±,78^{c}	48±2,74^{ab}
3	37±2,58^{a}	44±4,92^{bc}	54±3,45^{c}	43±2,74^{ab}
4	32±2,58^{a}	40±6,32^{b}	50±2,68^{c}	35±4,47^{b}

Note: Different letters in the same row indicate significant differences (P<0.05) and the same letters in the same row indicate insignificant differences (P>0.05).

The results showed that the addition of red watermelon extract to egg yolk tris dilution was able to maintain the quality of spermatozoa at 50C. Progressive motility of spermatozoa of kacang goat at 50C storage decreased according to storage time. Spermatozoa motility has decreased due to the energy source needed by spermatozoa in diluent decreases according to the length of storage time.

The average progressive motility on day 0 of storage for P0 (Control), P1 (1% ESM), P2 (2% ESM), and P3 (3% ESM) was still quite high, between 73-58%. The motility rate at the start of storage is quite high because the nutrient content contained in all treatments is still sufficiently available. This gives an indication that the egg yolk added to this extract which contains lipoprotein and lecithin plays an important role in protecting spermatozoa against the effects of cold shock. The addition of tris aminomethan serves as a buffer to overcome the increase in pH. In addition, there is fructose which is needed in the process of metabolism so that the durability of spermatozoa in this diluent is better compared to other diluents. The average decrease in progressive motility of spermatozoa decreased significantly at P0 when compared to P1, P2, and P3 which contained ESM

The mean progressive motility of spermatozoa after storage on day 4 for P0, P1, P2, and P3 were 32±2.58%, 40±6.32%, 50±2.68%, and 35±4 respectively .47%. Progressive motility of spermatozoa on day 4 for P2 gave significantly different results (P<0.05) better than P0, P1, and P3. The addition of ESM of 2% (P2) can maintain the percentage of progressive motility above 50% which is good enough to be used for AI until day 4 of storage. The addition of ESM 1% (P1) and 3% (P3) of storage on day 2 and day 3 is already decreased below 50%. The high percentage of progressive motility of spermatozoa in the addition of ESM of 2% is probably due to the balanced content of vitamin C, beta-carotene and lycopene in ESM as antioxidants.

The content of vitamin C, beta-carotene, and lycopene can optimize the rate of fructolysis so that the energy requirements for motility and survival of spermatozoa can be met. Beta-carotene is a fat-soluble carotenoid which is a precursor to vitamin A. Beta-carotene is a strong antioxidant and can eliminate the effects of single oxygen. Elwinda et al. (2011) stated that the lycopene content in watermelon has better antioxidant power than vitamins C and E. Lycopene as an antioxidant has the ability to fight damage to body cells due to free radicals by reducing the effects of toxins from reactive oxygen species (ROS).

The content of vitamin C can neutralize hydroxyl radicals, superoxide, and hydrogen peroxide and prevent agglutination of spermatozoa, as antioxidants break the chain reaction, allowing for the regeneration of reduced vitamin E. Research by Astirin et al (2003) proved that the addition of vitamin C could improve spermatogenesis and quality of mice spermatozoa after administration of tobacco extract and the results of research by Purnawati (2006) stated that administration of tomato juice which has high levels of lycopene and vitamin C can increase the number of mice spermatozoa strains exposed to cigarette smoke.

The results of this study support previous research that the progressive motility of spermatozoa decreased according to the length of storage time. This is in accordance with what Onky et al. (2014) that the percentage of spermatozoa motility of PE goat's diluted using Ringer dextrose was 61.87%. Kurniawan et al. (2018) obtained an average motility of PE goats with Ringer dextrose diluent of 71.87%. Adrianto (2016) found that the average motility of sheep spermatozoa using rind juice and red watermelon was 76.25%.

The results of this study also proved a decrease in the percentage of progressive motility of spermatozoa with the addition of 1% and 3% ESM, namely on the 3rd and 4th observation days there was slow movement of spermatozoa. This phenomenon

occurs due to the negative effect due to the high and low calcium content found in the watermelon extract solution which affects the spermatozoa capacity of the kacang goat. The calcium content contained in P2 is ideal calcium to maintain spermatozoa during cold storage.

According to Johnson et al. (2000), high concentrations of extracellular calcium in the medium can rapidly decrease the motility and metabolism of spermatozoa due to increased levels of intracellular ions in the cells, whereas low intracellular ions in the diluent medium can stimulate cell metabolism. Changes in temperature and osmotic pressure can also affect the structure of the lipid composition of the spermatozoa cell membrane so that it can cause a decrease in spermatozoa motility (Chun-Xia and Zeng-ming 2000). Progressive motility studies obtained in this study were still in the normal range according to Garner and Hafez (2000), namely between 40% -75%.

- **Viabilitas Spermatozoa**

Examination of spermatozoa viability was carried out using smear preparations stained with eosin nigrosin. The results of the spermatozoa viability examination of the kacang goat are shown in Table 2 below.

The percentage of viability in the treatment without using ESM in diluents showed a lower percentage of viability of spermatozoa when compared to those using ESM as shown in the table below.

Table 2. Percentage of viability of kacang goat spermatozoa in watermelon (Citrullus lanatus) fruit extract at 50C storage for 4 days.

Storage time (H)	Treatment of watermelon fruit extract concentration (%)			
	0	1	2	3
0	78,2±2,23^{a}	84,3±1,75^{b}	90,8±1,47^{c}	85,2±1,17^{b}
1	76,8±1,17^{a}	82,5±1,38^{b}	87,5±0,84^{c}	82,7±1,63^{b}
2	73,8±1,17^{a}	79,8±1,72^{b}	84,8±1,47^{c}	79,7±1,21^{b}
3	70,3±1,86^{a}	77,0±2,61^{b}	82,0±1,79^{c}	74,5±4,46^{b}
4	58,2±4,96^{a}	67,2±3,19^{b}	68,3±3,27^{b}	69,2±1,17^{b}

Note: Different letters in the same row indicate significant differences (P<0.05) and the same letters in the same row indicate insignificant differences (P>0.05).

Spermatozoa viability showed that the addition of ESM to egg yolk tris diluent had a significant (P<0.05) effect on the percentage of live spermatozoa in kacang goats. The percentage of spermatozoa viability in the use of ESM was better than the P0 treatment without ESM. There was no significant difference between the P1, P2 and P3 treatments.

The results of the study proved that the addition of ESM in egg yolk tris diluent could maintain the composition and physiological conditions of the diluent, so that the percentage of viable spermatozoa could be maintained. This situation occurs because ESM contains lots of vitamin C, beta-carotene, and lycopene which act as antioxidants. The content of vitamin C, beta-carotene, and lycopene can optimize the rate of fructolysis so that the energy requirements for the survival of permatozoa can be fulfilled. In addition, the content of vitamin C, beta-carotene, and lycopene can bind free oxygen radicals contained in the diluent and spermatozoa cells so as to prevent the formation of lipid peroxides which can damage the plasma membrane of spermatozoa cells.

According to Comb (1992) vitamin C contained in ESM will soon turn into the ascorbyl radical which is very reactive to oxygen radicals and hydroxyl radicals. Furthermore, the addition of 200 mg/100 ml of diluent of vitamin C can act as a protector of the sperm plasma membrane of horse spermatozoa (Aurich et al., 1997) and Boer goat breeds (Akbar, 2009), from damage by lipid peroxide. The addition of ESM in egg yolk tris diluent in this study maintained the percentage of live spermatozoa in kacang goats.

The percentage value of live spermatozoa on day 4 decreased slightly, this was due to the composition of the watermelon fruit which only relied on carbohydrates with little fat and protein. As well as being a source of energy and also acting as an extracellular cryoprotectant for spermatozoa against cold shock at 3-5^{0}C (Arifiantini et al., 2009); Yunawati and Herdis, (2009),

so that on day 4 when the fructose content is used up for metabolism, the function of fructose in watermelon as an extracellular cryoprotectant for spermatozoa also decreases, but the percentage of its viability is still suitable for AI use.

The results of this study support previous research that the viability of spermatozoa decreased according to the length of storage time. This is in accordance with that obtained by Hariati (2019) that the viability of spermatozoa of local goats by administering turi leaf-based organic tablets was 54.62%. Adrianto (2016) the effect of peel and watermelon juice on sheep spermatozoa was 54.33%.

The spermatozoa of kacang goat in this study were of good quality so that these spermatozoa could be further processed for AI purposes. this is in accordance with the opinion of Kusumawati et al. (2016a) which states that one of the minimum standards for the quality of spermatozoa that can be used for AI is a minimum of 50% percentage of live (viability) and motile spermatozoa.

- **Abnormalitas Spermatoozoa**

Data on the average percentage of abnormal spermatozoa of kacang goat diluted in treatments containing various concentrations of ESM at 50C storage temperature can be seen in Table 3 below.

Table 3. Percentage of abnormalities

Storage time (H)	Treatment concentration (%)			
	0	1	2	3
0	8,5±1,38^{c}	6,8±0,75^{b}	2,3±0,52^{a}	8±0,63^{c}
1	9,8±1,60^{c}	7,5±0,83^{b}	3,0±0,63^{a}	9,2±0,75^{c}
2	11,3±1,51^{c}	8,8±0,41^{b}	4,2±0,75^{a}	10,2±1,17^{c}
3	12,3±1,03^{c}	10,2±0,98^{b}	5,5±1,05^{a}	11,0±0,89^{b}
4	13,8±0,75^{c}	11,3±0,82^{b}	6,8±1,17^{a}	12,3±0,82^{b}

Note: different letters in the same row indicate significant differences (P<0.5) and the same letters in the same row indicate insignificant differences (P>0.5).

The results of statistical analysis showed that storage time had a very significant effect (P<0.5) on spermatozoa abnormalities. Table 3 shows that the percentage of abnormal spermatozoa has increased with increasing storage time. According to Suyadi et al. (2015) that the increase in abnormalities is caused by a pleated peroxide process, changes in osmotic pressure due to free radicals and lactic acid resulting from metabolic processes, thus damaging the plasma membrane and causing an increase in spermatozoa abnormalities. In addition, Yani el al. (2001) stated that the longer the storage time, the higher the percentage of abnormalities caused by stress, cold, and an imbalance in osmotic pressure as a result of ongoing metabolic processes.

The average percentage of abnormal spermatozoa of kacang goat on 4 days of storage ranged from 2.3%-13.8%. The treatment added with ESM was able to reduce abnormalities in the spermatozoa of kacang goat compared to the treatment without ESM. The control treatment without ESM was (13.8%) significantly different (P>0.5) by giving 1% ESM (12.3), giving 2% ESM (6.8%) and giving 3% ESM (12.3 %). The use of 2% ESM had a better effect than all treatments on goat spermatozoa abnormalities stored at 5oC.

Abnormalities observed in this study included broken tails or heads, twisted tails, and coiled tails. Crooked tails were the most common spermatozoa abnormality found at the time of observation when compared to tails or severed heads and coiled tails. The large number of bent or broken tails that were found was probably caused by the way the smear preparations were made or when the sperm was mixed with the diluent in the test tube, shaking too hard could affect the abnormalities of the spermatozoa. This is in accordance with the opinion of Toelihere (1991) that shaking the sperm or making preparations for a smear that is not proper can cause the head of the spermatozoa to break off or separate from the tail.

The results of this study can still be used for AI because it is still within the normal standard range for spermatozoa abnormalities. This is in accordance with the opinion of Ax et al. (2008) that the standard percentage of abnormal spermatozoa in goats that is suitable for AI is not more than 15%. According to Kartasudjana (2001) abnormal sperm for AI purposes should not contain more than 20% abnormal spermatozoa.

IV. CONCLUSIONS

1. The addition of yellow watermelon fruit extract to tris dilution of chicken egg yolk in maintaining the quality of spermatozoa of goat kacang at 5^0C storage showed better results for P2 progressive motility of 50%, viability of 68.3% and abnormality of 6.3%.

2. The addition of red watermelon fruit extract with a concentration of 2% is the optimal concentration in maintaining the quality of spermatozoa of kacang goat at 5^0C storage temperature.

RECOMMENDATIONS

1. Further research is needed to determine the fertility level of spermatozoa of kacang goat using various diluents added with yellow watermelon extract.

2. It is necessary to carry out further research on the effect of adding yellow watermelon extract on the quality of spermatozoa of kacang goat using different concentrations.

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