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by Daud Samsudewa

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Research Article

Optimization of Semen Diluents Using Filtration Technique Enriched with *Indigofera sp. Leaf extract*

Yon Soepri Ondho*, Enny Tantini Setiatin, Daud Samsudewa, Sutiyono, Alam Suryawijaya and Dela Ayu Lestari

Department of Animal Science, Faculty of Animal and Agricultural Sciences, Diponegoro University, Semarang, Central Java, Indonesia
***Corresponding author:** yon_supriondho@yahoo.com

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ABSTRACT

Nowadays, semen diluents that commonly used has high turbidity and it can decrease the semen quality. A renewable formulation for semen diluents which is clear and has low turbidity is necessary. Objectives of this study were to formulate a clear semen diluents using filtration technique without reducing any requirements and functions of the semen diluent and to analyze the microscopic quality of the semen diluted with and without filtration. The material used was fresh semen that collected from 8 Ettawa Grade bucks that were collected two times in a week for 1 month, and semen diluents that made of stock solution (chemical formulations) containing *Indigofera sp*. leaf extract. The equipment used were one unit of semen quality test, membrane filters, and turbidity meter. Parameters observed were the level of turbidity–measured by testing the turbidity of filtered semen diluents (FSD) and non-filtrated semen diluents (NFSD) and the quality of semen diluted by both diluents comprising progressive motility, viability, and abnormalities. The result of the study showed that semen diluents produced was clear (turbidity levels = 26.96 Nephelo Turbidity Unit) by using filtration techniques. Under microscopic observation, the quality of semen was 57.44% of progressive motility, 430 minutes of viability, and 31% of abnormality, which it was still above the normal standard (more than 20%). In conclusion, clear goat semen diluent was produced and had a good effect on progressive motility, viability and abnormality of spermatozoa cell.

Key words: Diluents, Filtration, *Indigofera sp*., Stock solution, Turbidity

INTRODUCTION

Semen diluents must have a good level of clarity and a long retention ability without reducing the quality of the semen to fertilize. However, the fact showed that most of semen diluents physically and visually have high turbidity (Bresciani *et al*., 2013). Turbidity becomes higher when the fresh semen is diluted into turbid diluent and in other side turbidity also related with the concentration of the plasma protein of spermatozoa cells. This turbid condition can decrease the semen quality and becomes an obstacle on evaluating proses of diluted spermatozoa cells, especially in microscope observation. Therefore, a technique that can reduce the turbidity of semen diluents is seriously needed.

In present, there are at least 3 semen diluents with a clear form called *AndroMed*, *Bioxell and optiXcell* (Ansari *et al*., 2017; Akhter *et al*., 2011) that have been introduced and marketed in Indonesia. *AndroMed,* and *Bioxell* have been applied by most of frozen semen factories in Indonesia. Meanwhile, local diluents products composition were colloidal ingredients based (yolk and skim milk).

Skim milk and egg yolk have been used as they have proven to be the best foundation materials of semen diluents (Susilowati, 2017); although, its appearance is still turbid. This turbidity appears due to the formation of colloidal suspensions and large particle that generated from semen diluent material (Mostafapor and Ardebili, 2014). However, the large particles could be filtered into smaller ones, so the turbidity of the semen diluent might change to be clearer (Oxtoby, 2002). By reducing the large number of particles in the diluent, the spermatozoa cells could utilize nutrients in the diluent to increase their fertilization and preservation intensity.

In general, direct filtration to the diluents that contain Tris egg yolk or skim milk (suspense/colloidal) is difficult to produce good clarity of the diluent. Because those two

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materials have high turbidity, as they have big size of particles (Buckle *et al*., 1987). Study by Amer *et al*. (2008) concluded that the filtration and centrifugation treatment on Tris egg yolk and skim milk before dilution resulted in good spermatozoa quality. To approach this problem, filtration method could be applied to reduce particles size of the diluents materials (Scott, 1995); so that, the filtering stage can be conducted easily to produce clear diluents.

Given this situation, the aims of this study were to formulate clear semen diluents using filtration technique without reducing any requirements and functions of the semen diluent and to analyze the microscopic quality of the semen diluted with and without filtration. It is expected that the result of this research might provide new approach to semen diluent purification technique.

MATERIALS AND METHODS

Ethical approval

This study was approved by the Animal Ethics Committee on the use of Animal and Scientific Procedures at the Faculty of Animal and Agricultural Sciences, Diponegoro University, Indonesia.

Research location

The research was conducted at the Laboratory of Genetics, Breeding, and Animal Reproduction and the Laboratory of Nutrition and Animal Feed, Faculty of Animal and Agricultural Sciences, Diponegoro University from October to November 2018. Semen collection was carried out at stalls owned by Faculty of Animal and Agricultural Sciences, Diponegoro University.

Research materials, equipment and tools

The research materials consisted of semen that collected from eight Ettawa Grade bucks, chemical substances composing stock solution of semen diluent (Table 1) and *Indigofera sp*. leaf extract. The supporting materials used were 2% eosin, aquabidest, KJ Jelly, and 70% alcohol. Meanwhile, the tools used was service crate and artificial vagina to collect semen, microscope, deck-glass/cover-glass, and steam bath to analyze semen quality; electric scale, beaker glass, incubator, Erlenmeyer, test tube, micropipette, scale pipette, stopwatch and incubator to process the semen diluent; and SIGMA membrane filter (Durapore Polyvinylidene) to filter the semen diluent, centrifuge and turbiditymeter.

Formulation methods

Semen diluents preparation

The chemical substances for making stock solution from chemicals substances were presented in Table 1. Chemical substances of A.1, 2, 3, 6, then B.1 and 2, were accurately weighed and poured into 100 ml Erlenmeyer. After adding 70 ml of aquabidest to the Erlenmeyer, the solution was homogenized using magnetic stirrer. Chemical substances of A4 and A5 were carefully weighed and poured into 25 ml Erlenmeyer. After adding 25 ml of aquabidest to the Erlenmeyer, the solution was homogenized using magnetic stirrer. The solution 2 was poured drop by drop into solution 1 and then add aquabidest to the Erlenmeyer until reaching 100 ml and add 1000 IU penicillin. One hundred ml stock solution was available.

Preparation of *Indigofera Sp.*

Three grams of fresh *Indigofera sp*. leaves were added with 100 ml of aquabidest, then blender them until becoming pulp. The pulp was put into a centrifuge and run at 7500 rpm for 10 minutes (Taulbee and Mercedes, 2000) until the pulp was separated into two *Indigofera sp*. extracted parts; supernatant at the top and substrate at the bottom. The supernatant was taken and put in the centrifuge tube, then it run at 7500 rpm for 10 minutes until the supernatant became clearer.

Adding the extraction of *Indigofera sp*. into the etock solution

One milliliter of the supernatant of the *Indigofera sp*. leaves extraction was added to 100 ml of stock solution, then homogenized using a magnetic stirrer. The result of a 100 ml semen diluent was divided into 2 parts, each of which was 50 ml and put them into Erlenmeyer.

Filtration treatment of the diluent

The two parts of the semen diluent each of which was 50 ml were treated differently. The first part was not filtrated and the second one was filtrated. The filtration technique was conducted by sucking the 50 ml semen diluent into a 10 ml size of disposable syringe through its needle. Then, the needle was released and a filtrator having a pore diameter of 0.22 μm was attached to the tip of the syringe. Both the filtrated and non-filtrated semen were put into tightly closed bottles and temporarily stored in the cool box before they were used.

Collecting and examining the quality of the fresh semen

Eight Ettawa Grade bucks were selected based on age 2.5 - 3 years and in healthy condition. Their semen was collected two timeSs in a week for 1 month. According Toelihere (1993) fresh semen were examined macroscopically (volume, pH, viscosity, color and smell) and microscopically (mass motility, progressive motility, viabilities/survival rate, abnormalities, and concentrations). The result of the observations on the quality of the fresh semen were used as a cross reference for non-filtrated semen diluents (NFSD) and filtrated semen diluents (FSD).

Research treatment

The fresh semen was diluted with NFSD and FSD. Each treatment was replicated 8 times and the results of each replication were put in the tube then was stored in the incubator (temperature 37.5^{0}C). The observation was conducted microscopically to analyze the progressive motility, semen viability and abnormality.

Parameters observed

A. **Turbidity levels of semen diluents**

The observation of the turbidity levels was conducted by measuring the turbidity levels using a portable digital turbidity meter 0-200 NTU. The results of the NFSD and FSD then were compared. The turbidity of the diluent was expressed in NTU (Nephelometric Turbidity Unit).

B. **Quality of semen:** The semen quality was observed, those were:

(1) Progressive Motility, the percentage of sperms progressive motility.

(2) Semen viability, the living spermatozoa cells was calculated periodically in 30 minutes until their viability decreased under 10%.

(3) Abnormalities, the sperms were analyzed by observing the occurrence of the secondary abnormalities due to the dilution process and the occurrence primary abnormalities by comparing to the fresh semen. Then, the results were compared.

Data analysis

The data of filtrated and non-filtrated semen clarity were compared and analyzed descriptively; while, the data of the quality parameter were analyzed using T-Test (Gasperz, 1989; Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Fresh semen

The result of the macroscopic and microscopic examination on average fresh semen quality (Ettawa Grade Goats) were summarized in Table 2. The result of the fresh semen tested microscopically showed that all parameters met the standard requirement. Fresh semen quality parameters that observed macroscopically (volume, smell, color, pH and viscosity) and microscopic (mass motility, progressive motility, concentration, viability) is qualify for dilution (Toelihere, 1993; Ax *et al*., 2000)

Diluents

The results of the turbidity test showed that FSD and NFSD had turbidity as much as 26.96 and 47.06 NTU, respectively. This suggested that NFSD contained suspensions/colloids of particles with diameter more than 0.22 μm and in large quantities. Therefore, NTU value of NFSD was higher (Table 3) compared to FSD and the appearance of the FSD was clearer than NFSD.

The appearance of the semen diluent formulated from stock solution enriched with *Indigofera sp*. was illustrated in Figure 1. The appearance of NFSD has suspended particles and greenish colors. These particles were formed from the bond of materials composing semen diluents (Peruma, 2018) while, the greenish was the existing chlorophyll even after the leaves of the *Indigofera sp*. had undergone a centrifugation process to obtain the supernatant.

The results of the turbidity test showed that the NFSD had 47.06 NTU on average. This indicated that the semen diluent contained suspensions/colloids that formed particles with diameter of greater than 0.22 μm and in large quantities. As a result, the NTU value was higher (Table 3) compared to FSD. The appearance of the filtrated semen diluents enriched with *Indigofera sp*. was illustrated in Figure 2. The appearance of the FSD was clear (the level of clearness is 26.96 NTU). The advantages of the clear semen diluents were facilitating the microscopic observation such as motility, viability and abnormality.

Table 1: Chemical substances composing stock solution of semen diluent

Part	Chemical Materials	Concentration (g/100ml)
A.1	NaCl	0.6546
2	KCl	0.0300
3	$NaH_2PO_4.6H_2O$	0.0228
4	$MgCl_2.2H_2O$	0.0106
5	CaCl2.2H2O	0.0331
6	NaHCO3	0.3108
7	Penicillin (IU)	1000
B.1	Glucose	0.2756
2	Na Pyruvate	0.0138

Stock solution is modified made by modification of the D-PBS medium (Hafez, 1993[b]).

Table 2: Macroscopic and microscopic quality of fresh semen

Parameter	Average
Macroscopic	
Volume (ml)	1.17±0.67
Smell	Spermin
Color	Milky White
pH	6.8
Viscosity	Relatively consistence
Microscopic	
Mass Motility (score)	(++)
Concentrations (10^7)	220,20
Abnormality (%)	19.20
Progressive Motility (%)	66.7
Viability in open chamber (minutes)	19.2

Table 3: Turbidity test of fresh semen.

Replication	Turbidity (NTU)	
	NFSD	FSD
1	47.50	27.40
2	46.90	26.70
3	46.80	26.80
Average	47.06[a]	29.96[b]

[a, b] = different superscript letters in each raw is significantly different. NFSD=Non-Filtrated Semen Diluent; FSD =Filtrated Semen Diluent; NTU=Nephelo Turbidity Meter.

Semen quality

Progressive motility

Result of progressive motility observation between fresh semen and diluted semen with NFSD and FSD was presented in Table 4. The motility of the spermatozoa cells in the diluted semen of NFSD was decreased to 13% compare to fresh semen. It was due to the high turbidity of the semen diluents (47.06 NTU), the progressive motility is influenced by physical properties of the semen diluents (Miki, 2007). In other word, physical properties that inhibit the progressive motility of spermatozoa cells were chemical ingredients in the form of large particles of suspensions or colloids. Meanwhile, the progressive motility of the spermatozoa cells in the FSD was better than NFSD (50% vs 55%). By filtering the diluent, the inhibitor of the progressive motility with size more than 0.22 μm was reduced.

Viability of the spermatozoa cells

Viability of the spermatozoa cells observation was conducted by examining the spermatozoa cells viability in FSD and NFSD as shown in Table 5. Result showed that the average viability of the spermatozoa cells in the NFSD was 379 minutes (6 hours 19 minutes) compared to fresh semen (15 minutes). Spermatozoa cells had longer viability due to the availability of adequate nutrition in the

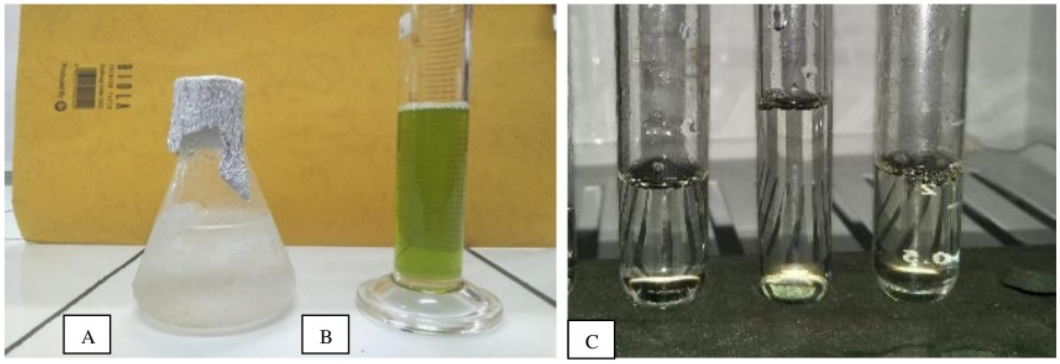

Fig. 1: The appearance of the semen diluent consisting of stock solution enriched with *indigofera sp*. (A = stock solution; B= extract of *indigofera sp*.; C = stock solution enriched with *indigofera sp*).

Table 4: Progressive motility of spermatozoa cell in fresh semen, semen diluted with NFSD and FSD

Buck	Progressive Motility (%)		
	Fresh Semen	NFSD (47.06 NTU)	FSD (26.96 NTU)
1	68	50	60
2	65	45	45
3	68	50	60
4	65	55	60
5	68	50	55
6	66	55	60
7	67	50	55
8	65	45	50
Average	66.5	50	55.6

NFSD=Non-Filtrated Semen Diluent; FSD=Filtrated Semen Diluent.

Table 5: Viability of spermatozoa cell in fresh semen, semen diluted with NFSD and FSD

Buck	Viability (minutes)		
	Fresh Semen	NFSD	FSD
1	20	390	400
2	19	375	480
3	20	400	420
4	17	350	450
5	20	380	400
6	18	385	460
7	16	380	450
8	17	375	430
Average	18.3	379.3[a]	436.2[b]

[a, b] = different superscript letters in same raw is significant different (P<0.01); NFSD=Non-Filtrated Semen Diluent; FSD =Filtrated Semen Diluent.

Table 6: Abnormality of spermatozoa cell in fresh semen, semen diluted with NFSD and FSD

Buck	Abnormality (%)		
	Fresh Semen	NFSD	FSD
1	18	35	35
2	20	37	33
3	17	30	30
4	21	35	25
5	20	34	30
6	19	35	30
7	17	30	30
8	18	35	30
Average	18.7	33.8	30.7

NFSD=Non-Filtrated Semen Diluent; FSD =Filtrated Semen Diluent.

Fig. 2: A= Filtering process of semen diluents enriched with *Indigofera Sp*.; B= filtering results.

diluents, adjustment of ambient temperature and controllable humidity to avoid excessive evaporation of liquid; so that, the spermatozoa cells were at isotonic condition and stable pH diluents. The level of turbidity of the diluent influenced the input rate of carrying nutrients of diluents into spermatozoa cells through cell membrane (Hafez, 1993[a]).

In general, cells utilized external nutrient by diffusion process in cells membrane (Alberts *et al*., 2000). The membrane diffusion process is influenced by particles size due to suspension/colloid in both extra cell and intra-cell fluids (Nikaido and Rosenberg, 1981). Furthermore, as shown in Table 5, spermatozoa cells in FSD proved having longer viability than NFSD (430 minutes vs 379 minutes). In FSD, spermatozoa cells were easier and faster to utilize smaller and softer particles nutrients than in NFSD. The small and soft size particles (0.22 μm) caused spermatozoa membrane cells easily absorbing the compound/colloid suspension based on nutrients from extra cells to intra cells. Therefore, the viability of the spermatozoa cells was longer.

Abnormalities of spermatozoa cells

Data on the spermatozoa abnormality in both fresh semen, semen diluted in NFSD and FSD were presented in Table 6. In this observation, the abnormality was categorized as primary abnormalities in fresh semen and secondary abnormalities in diluted spermatozoa cells. Result showed that the abnormality of fresh semen

(19.2%) was better than the minimum requirement of good semen (20%). This abnormality was categorized as primary abnormality, because it occurred since the process of spermatogenesis until ejaculation (Parimal and Anil, 2011; Arifiantini *et al*., 2010). As the fresh semen was diluted with NFSD, the abnormality increased by 34.43%. Suspension-colloidal properties contributes to the collision taking place in the semen diluents; a physical collision of spermatozoa cells with each other (Myre and Shaw, 2006). Microscopic observation showed that the secondary abnormality appeared by the presence of broken tail parts of spermatozoa, cracks in the cell membrane of spermatozoa, and others.

The percentage of the spermatozoa cell abnormality in FSD was lower than that of in NFSD (31% vs 34.43%). In FSD, the frequency of physical collision events between spermatozoa cells is lower and weaker than in NFSD. Although the finding showed that the abnormality of the FSD was 31%, Parimal and Anil (2011) argued that, in certain cases, 40% of sperm abnormality is in tolerable range. Other finding showed that the abnormality of the spermatozoa cells in both NFSD and FSD was relatively high, as the semen diluents formulation consisted of materials supported cell viability.

Conclusions

This research has successfully formulated clear semen diluents, having turbidity of 26.96 NTU by enriching stock solution with 1% *Indigofera Sp* and filtration technique. The semen quality test conducted microscopically proved that semen diluted with this semen diluents had optimum progressive motility, high percentage of viability, and percentage of spermatozoa cells abnormality above normal (more than 20%). Therefore, in regard to this successful finding, further research might also be suggested by putting emphasis on reformulation of the materials composing semen diluents.

REFERENCES

Akhter SI, MS Ansari, BA Rakha, N Ullah, SM Andrabi and M Khalid, 2011. In vitro evaluation of liquid-stored buffalo semen at 5°C diluted in soya lecithin based extender (Bioxcell®), tris-citric egg yolk, skim milk and egg yolk-citrate extenders. Reprod Dom Anim, 46 (1): 45-49.

Alberts B, A Johnson and J Lewis, 2002. Transport into the Cell from the Plasma Membrane: Endocytosis. In: Molecular Biology of the Cell. 4th ed. Garland Science, New York.

Amer H, H Mansour, A El-Sayed and A Swelum. 2008. Influence of filtration or centrifugation of semen diluent on the viability of chilled buffalo-bull semen. Stocarstvo, 62: 351-366.

Ansari MS, BA Rakha and S Akhter, 2017. Cryopreservation of bull semen in OptiXcell and conventional extenders: Comparison of semen quality and fertility. Anim Scie Papers Reports, 35: 317-328.

Arifiantini RI, B Purwantara and M Riyadhi. 2010. Occurrence of Sperm Abnormality of Beef Cattle at Several Artificial Insemination Centers in Indonesia. Animal Production, 12 (1): 44-49.

Ax RL, M Dally, BA Didion, RW Lenz, CC Love, DD Varner, B Hafez and ME Bellin. 2000. Artificial Insemination. In: Reproduction in Farm Animals. Edited by B. Hafez and ESE. Hafez. 7th ed. Lippincott Williams and Wilkins, Philadelphia, pp: 424-459.

Bresciani C, G Morini, R Bettini, E Bigliardi, FD Ianni, CS Cabassi, A Sabbioni and E Parmigiani. 2013. Reproductive efficiency of a new modified boar semen extender for liquid storage. Livestock Sci, 157(1): 384–388.

Buckle KA, RA Edwards, GH Fleet and M Wotton. 1987. Ilmu Pangan. (Penerjemah Hari Purnomo dan Adiono). Universitas Indonesia Press, Jakarta.

Gasperz V. 1989. Metoda Perancangan Percobaan. Penerbit CV Armico, Bandung.

Hafez ESE. 1993[a]. Semen Evaluation. In: Reproduction in Farm Animals. 6th ed. Edited by ESE Hafez. Lea and Febiger, Philadelphia, pp: 405-423.

Hafez ESE. 1993[b]. Preparat of Physiologic Solutions (Appendix IV). In: Reproduction in Farm Animals. 6th ed. Edited by ESE Hafez. Lea and Febiger, Philadelphia, pp: 543-544.

Miki K. 2007. Energy metabolism and sperm function. Social Reproduction Fertility Supplement, 65: 309-325.

Mostafapor S and FF Ardebili, 2014. Effects of diluting medium and holding time on sperm motility analysis by CASA in ram. Veterinary Research Forum, 5(2): 101–105.

Myre E and R Shaw, 2006. The Turbidity Tube: Simple and Accurate Measurement of Turbidity in the Field. Michigan Technology University, Michigan.

Nikaido H and EY Rosenberg. 1981. Effect on solute size on diffusion rates through the trans membrane pores of the outer membrane of Escherichia coli. Journal of Genetic Physiology, 77 (2):121-135.

Oxtoby DW. 2002. Prinsip-prinsip Kimia Modern. Jilid 1. Edisi 4. Erlangga. Jakarta.

Parimal MP and SS Anil. 2011. Sperm abnormalities and its treatment. Int J Pharm, 2(11): 46-49.

Peruma P, 2018. Low density lipoprotein in cryopreservation of semen. Asian Pacific J Reprod, 7: 103-116.

Scott K. 1995. Microfiltration. In: Handbook of Industrial Membranes. 1st edition. Elsevier Advanced Technology, UK.

Steel RGD and JH Torrie. 1980. Principles and Procedures of Statistics, A Biometrical Approach. 2nd edition. McMgraw Hill, Aukland.

Susilowati T, 2017. Spermatology. Cetakan Kedua. Universitas Brawijaya, Malang.

Taulbee DN and MMV Mercede. 2000. Centrifugation. University of Kentucky-Center for Applied Energy Research, USA.

Toelihere MR. 1993. Inseminasi Buatan Pada Ternak. Penerbit Angkasa, Bandung.

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