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Development of technology production of frozen of swamp buffalo (*bubalus bubalis*) in the kampar regency

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ABSTRACT

The objective of this study was improve the quality of Kampar buffalo spermatozoa during the cryopreservation process. The diluters were Tris yolk standards, commercial thinners concentrate (Triladyl®), and plant-based commercial diluent, soybean lecithin (Andromed®). Sperm was collected from artificial vagina. The parameters observed were the motility, live spermatozoa, plasma membrane integrity (PMI) pre and post-freezing after incubation. The treatments consisted of four doses of semen ($10x10^6$ sperm/ml, $15x10^6$ sperm/ml; $20x10^6$ sperm/m, and $25x10^6$ sperm/ml), each consisted of three replications. The data were analysed by analisa of variance 3x4x4 factorial. The result showed that the Male buffalo in Kampar regency has a decent semen for semen freezing. Motility, percentage of live sperm and PMI on the use of Andromed® as a diluent in different buffalo sperm concentration is better when compared with Triladyl® and Tris yolk standards. The use of after equilibration semen diluent Andromed® in frozen semen of mud buffalo gave higher PMI when compared to Triladyl® and Egg Yolk Tris. Kampar buffalo semen motility post-tired at all three different types of diluent was not different while the live percentage and PMI frozen buffalo semen post-tired on the use of diluent Andromed® was higher than the use of diluent Triladyl® and Tris Egg Yolk. However Andromed® is a diluent which is superior in providing protection to the sperm during the freezing process.

Keywords : kampar buffalo, equilibration, cryopreservation

INTRODUCTION

Application of Artificial Insemination (AI) with frozen-thawed semen has been reported at limited scale in buffalo, because of poor freezability and fertility of buffalo bull spermatozoa as compare to cattle bull spermatozoa (Nazier, 2001; Ahmad et al., 2003; Kumaresan et al., 2005). Although AI has been practiced for the past 50 years, fertility rate with this technology is less and unpredictable in buffaloes (Agarwal and Tomer, 2003). Semen extenders used in these trials contained egg yolk, which carries a certain hygiene risk (Hartmann et al., 1998; Mu¨ller-Schlo¨sser et al., 2001). Some researchers adopted from the international literature and the other developed at regional and local level (Vale, 2010). The main aim of this study was to evaluate formula for semen diluent in the processing and manufacture of frozen semen of Kampar regency's mud buffalo through in vitro assays and to compare the protective power (cryoprotectivity) of egg yolk-based diluent to a diluent which is not based on egg yolk as materials for frozen buffalo semen of Kampar regency's mud buffalo through in vitro assays..

MATERIALS AND METHODS

Experimental animals used as a source of semen were three adult male buffaloes, placed in individual cages belonged to the farmers. Natural grass around the farm was given. Additional feed given were bran and concentrate. The drink was given by *ad libitum*. This research was using a completely randomized design with 3 x 3 factorial and thrice replication. The factors were the type of Tris-egg yolk diluent conventional standards, commercial thinners concentrate (Triladyl®), and plant-based commercial diluent, soybean lecithin (Andromed®). The treatments consisted of four doses of semen ($10x10^6$ sperm/ml, $15x10^6$ sperm/ml; $20x10^6$ sperm/m, and $25x10^6$ sperm/ml), each consisted of three replications. The data is analyzed by using variation of investigation. The difference between the average among the three diluents were compared by using ANOVA (the General Linear Models Procedure/GLM for the least square means of SPSS 10) (Steel and Torrie, 1991). The Variables measured were motility, live spermatozoa, plasma membrane integrity (PMI) pre and post-freezing after incubation.

The diluent was prepared 1 day prior to the shelter. Semen accommodating was made using an artificial vagina which was filled hot water (40-45°C) using a teaser (angler). Each buffalo was scheduled for semen accommodating 2 times per week at the same interval (Monday and Thursday) in the morning (6:00 to 8:00 pm). Each buffalo left to serve artificial vagina once per accommodating in 5 minutes after false mount. Accommodated semen in the glass was immediately taken to the laboratory for semen processing and stored in a water bath (37°C) for further testing or assessments carried out a variety of motility and other semen characteristics (macroscopic and microscopic examination).

RESULTS AND DISCUSSION

Quality of Fresh Sperms. The average of motility percentage at the first ejaculation of the three males was 75% (Table 1). Semen volume varied among the three males buffaloes with an average range of 1.5 to 2.5 cc in the first and the second ejaculate. The results showed that the number of spermatozoa ranged between 900 and 1,500 with an average $1,200x10^6$ cells per ml. The average motility of semen in the third study males was higher than the first and second study. These results are not much different from Kustono (1992) who did the accommodating semen of Murrah buffaloes in Manila. The results of this study wass higher compared to Mukesh et al. (2010) who obtained buffalo semen motility of 71.42%, 58.2% and 44.62±0.02-47.08±0.05. This difference is thought to be caused by several factors such as season, stress and the study (Narinder, 2005). Concentration of Swamp buffalo in Kampar regency still within the range of research results of Kustono (1992) in Murrah buffaloes.

Quality of the Sperms After Freezing. The average of motility, percentage of live sperm and PMI on various types of diluent in different sperm concentrations can be seen in Table 2. The use of frozen semen diluent Andromed® in frozen semen of mud buffalo gave higher PMI when compared to Triladyl® and Egg Yolk Tris (Table 3). Motility, percentage of live sperm and PMI are the parameters used to determine the effect of experimental procedures on the storage of buffalo bull semen (Akhter et al., 2008, Andrabi et al., 2008). It is well recognized that sperm motility is affected by the properties of diluting media (Andrabi, 2009). In our study motility, percentage of live sperm and PMI on the use of Andromed® as a diluent in different buffalo sperm concentration is better when compared with Triladyl® and Tris yolk standards. This is probably caused by the content of soybean lecithin in Andromed® with low high-density lipoprotein (HDL) and is not like the yolk that inhibits respiration and

spermatozoa motility (Moussa et al., 2002). Motility, percentage of live spermatozoa and the PMI in this study differ from Triwulanningsih et al. (2011) which states that the use of standards with the addition of tris glutathionin further enhance sperm motility and percentage live in mud buffalo and Kustono (1992) who stated that the thinners tris egg the most efficient yolk keeping motility of Murrah buffalo's spermatozoa.

Motility, Percentage of Life and PMI Buffalo Semen after Equilibration

Motility and live percentage of buffalo semen after equilibration did not show different results in all three types of diluent (Tris, Triladyl® and Andromed®)(Table 3). The low value of motility, percentage of live spermatozoa and semen PMI after equilibration on the use of diluent Triladyl® and Tris Egg Yolk compared Andromed® allegedly because Andromed® contains steroid hormones and precursors (Hartmann et al., 1998). These hormones can cause a decrease in quality (Mu¨ller-Schlo¨sser et al., 2001). Motility and percentage live semen of buffalo in Kampar regency after equilibration higher than the African buffalo semen collected from the epididymis by using a diluent Triladyl® and Andromed® at several different times of equilibration (Herold et al., 2006). This difference is probably caused by different types of water buffalo and the different way of semen collection (Hafez, 2000).

Motility, Live Percentage and PMI of Buffalo Semen Post-Fatique

Post-fatique at all three different types of diluent was not different while the live percentage and PMI frozen buffalo semen post-tired on the use of diluent Andromed® was higher than the use of diluent Triladyl® and Tris Egg Yolk (Table 4). The similarity values of all three types of motility is due to the composition of the diluent buffers, nutrients (fructose) and egg yolk as an anti-cold shock as well as glycerol is almost the same. However Andromed® is a diluent which is superior in providing protection to the sperm during the freezing process (Arifiantini and Yusuf, 2004). Andromed is recommended to use in diluting and freezing buffalo semen. It needs to test frozen semen that has been made in female buffalo in the Kampar regency.

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Table 1. The average volume; sperm motility and concentration of Swamp Buffalo.

Stud	Accommodating	Volume of ejaculate (ml)		Motility (%)		Concentration (10^6/ml)	
1	1	1.5	1.5	1.5	70	70	900
	2	1.5	1.5	1.5	70	70	970
	3	1.5	1.5	1.5	70	70	890
	4	2	2.5	2.5	75	70	970
	5	2	2	2	75	70	960
	6	2	2	2	75	70	1000
2	1	2	2	2	75	75	1300
	2	2.5	2.5	2.5	75	75	1270
	3	2	2.5	2.5	75	75	1450
	4	2	2.5	2.5	75	75	1200
	5	2.5	2.5	2.5	75	75	1300
	6	2.5	2.5	2.5	75	75	1300
3	1	2	2	2	70	70	980
	2	1.5	2.5	2.5	70	70	1500

3	2	2	2	70	70	1500
4	2.5	2.5	2.5	70	70	1450
5	2.5	2.5	2.5	70	75	1500
6	2.5	2.5	2.5	75	75	1500

Table 2. Semen quality in various stages of dilution and spermatozoa concentration

Parameters	Type of Diluent	Spermatozoa Concentration x 10^6 cells/ml			
		10	15	20	25
Motilitas	Standard Egg Yolk Tris	71^a	72^a	70^a	70^a
	Triladyl®	71^a	70^a	71^a	71^a
	Andromed®	74^b	74^b	74^b	75^b
Percentage of Live Spermatozoa	Standard Egg Yolk Tris	76^a	76^a	76^a	77^a
	Triladyl®	76^a	76^a	76^a	76^a
	Andromed®	77^b	77^b	77^b	78^b
PMI	Standard Egg Yolk Tris	78^a	78^a	78^a	78^a
	Triladyl®	78^a	78^a	79^a	78^a
	Andromed®	80^b	80^b	80^b	80^b

Table 3. Semen quality in various stages of dilution and the concentration of spermatozoa after equilibrasi

Parameters	Type of Diluent	Spermatozoa Concentration x 10^6 cells/ml			
		10	15	20	25
Motility	Standard Egg Yolk Tris	68^a	68^a	68^a	68^a
	Triladyl®	68^a	68^a	68^a	69^a
	Andromed®	71^b	71^b	71^b	71^b
Percentage of Live Spermatozoa	Standard Egg Yolk Tris	71^a	71^a	71^a	71^a
	Triladyl®	72^a	71^a	71^a	71^a
	Andromed®	73^b	74^b	74^b	74^b
PMI	Standard Egg Yolk Tris	71^a	71^a	71^a	71^a
	Triladyl®	72^b	72^b	72^b	72^b
	Andromed®	73^c	73^c	73^c	73^c

Table 4. Semen quality in various stages of dilution and concentration of sperm after post-fatigue

Parameters	Type of Diluent	Spermatozoa Concentration x 10^6 sperma/ml			
		10	15	20	25
Motility	Standard Egg Yolk Tris	53^a	54^a	54^a	54^a
	Triladyl®	54^a	54^a	54^a	53^a
	Andromed®	55^a	55^a	55^a	54^a
Percentage of Live Spermatozoa	Standard Egg Yolk Tris	63^a	64^a	64^a	64^a
	Triladyl®	64^a	63^a	66^a	65^a

	Andromed®	67[b]	67[b]	67[b]	67[b]
PMI	Standard Egg Yolk Tris	61[a]	61[a]	61[a]	62[a]
	Triladyl®	63[b]	63[b]	63[b]	63[b]
	Andromed®	65[c]	66[c]	67[c]	67[c]

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