THE EFFECT OF EQUILIBRATION TIME ON THE QUALITY OF FROZEN SEMEN OF ETTAWA CROSSBRED AND BOER GOAT

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Abstract

One of the most important factors in processing of frozen semen is the time given for the sperms to adapt with cold temperature (equilibration time) before freezing take place. In the present study, semen of five heads of bucks from each breed, Ettawa Crossbred (PE) and Boer goats, was collected weekly. Good quality semen was diluted in Tris diluent at room temperature, and put into a ministraw. Thereafter, temperature was decreased slowly to 4-5°C within 60 minutes and it was maintained in this level for 1, 2 and 3 hours before freezing. The best-frozen semen obtained was used for artificial insemination. Results of the study showed that ejaculate volume of PE and Boer goats varied 0.3 - 1.5 cc/ejaculate, with motility score of 65-75% and live sperms of >70%. The second ejaculate showed better quality than those of the first ejaculate. The quality of frozen semen shown by motility (25-45%) and live sperms (35-65%) varied widely. Equilibration time (1-3 hours) significantly affected the quality of frozen semen, in which 3-hours equilibration time gave the best results. *In-vivo* fertility test of frozen semen of PE and Boer goats showed pregnancy rates of 31.43% and 31.25%, respectively. It can be concluded that 3-hours equilibration could give a better frozen semen quality compared with 1 and 2 hours, though *in-vivo* test showed a relatively low pregnancy rate.

Key words: Goat, Equilibration, Frozen semen, Pregnancy

Introduction

The utilization of a good genetic quality of bucks for breeding will be able to improve productivity of goat, and incorporation of artificial insemination (AI) technology into breeding program will increase the breeding efficiency of bucks (Wodzicka-Tomaszwescka *et al.*, 1993). At natural mating, each buck could mate 40 - 70 heads of does per mating season, while by AI the number of does inseminated increase to 700 - 1500 heads (Xiaowu, 1984). In-vitro studies showed that semen of PE goat has been successfully preserved in the form of frozen semen, and Tris-diluent was commonly used (Suwarso, 1998, Werdhany, 1999, Tambing *et al.*, 2000; Sutama *et al.*, 2000, 2001; Kostaman *et al.*, 2000). One of the most important factor in the process of frozen semen is equilibration time which is the time required for the sperms to adapt with cold temperature. Equilibration can prevent negative effect of glycerol and

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antibiotic present in the diluent, and reduced dead rate of sperm during freezing (Toelihere, 1985) and 4-6 hours equilibration time is recommended (Lindsay, 1982). Kostaman *et al.* (2000) found that 4 hours equilibration time gave better results than that of 2 hours equilibration. In general, however, there is no firm conclusion of the optimum time required for equilibration before freezing, and this is the focus of the present study.

Materials and Methods

Five heads of bucks from each breed of Ettawa Crossbred (PE) and Boer and 51 heads of mature PE does were used in this experiment. They were offered 4-5 kg forages and 0.5-0.7 kg concentrate (CP 15%) to meet nutrient requirement as recommended by NRC (1981). Semen of PE and Boer bucks was collected every week. A good quality semen (motility >70%, sperm concentration >2 billion/ml, live sperms >70%, abnormal sperms <10%) was diluted in Tris diluent until sperms concentration of 600 million/ml (Table 1). The diluted semen was put into a ministraw (0.25 ml/straw) at room temperature. Thereafter, temperature was decreased slowly to reach 4-5°C within 60 minutes and it was maintained in this level (equilibration) for 1, 2 and 3 hours before freezing.

The frozen semen was evaluated for motility and lives perms at 24 hours after freezing. The best frozen semen of PE and Boer goats were used for artificial insemination on 51 heads of PE does following oestrous synchronization using CIDR for 14 days. The non-return oestrous does were checked 15-25 days after insemination, by using vasectomies bucks. Data obtained were subjected to analysis of variance (Steel and Torrie, 1981).

Table 1. Composition of Tris diluent used in the experiment

Component	Quantity
Tris (*Hydroxymethyl amino methane*) (g)	2.96
Citric acid (g)	1.65
Fructose (g)	2.00
Egg yolk (ml)	20
Glycerol (ml)	6
Penicillin G (IUml)	1000
Streptomycin (μg/ml)	1000
Aquabidest (ml) ad.	100

Results and Discussion

Characteristics of Fresh Semen

The average value for ejaculate characteristics of PE and Boer goats is shown in Table 2. Ejaculate volume varied 0.3 - 1.5 ml/ejaculate, with motility score of 65-75%, live sperms of >70%, Sperm concentration >2 billion/ml, and abnormality <10%. In both breeds, the second ejaculate generally showed better quality than those of the first ejaculate.

On average, ejaculate volume of Boer was significantly lower compared than that of PE goats (0.68 vs. 1.2 ml, $P<0.05$) indicated breed effect. Some reports showed that ejaculate volume of Boer goat varied 1.2 - 2.03 ml (Igboeli, 1974; Greyling and Grobbelar, 1982; Tuli *et al.*, 1991), and ejaculate volume of PE goat was 0.5 – 1.7 ml (Tambing *et al.*, 2000, Kostaman *et al.*, 2000). Kacang goat with small body size had relatively high ejaculate volume (1.62 ml) (Soeparna, 1984), while Nubian goat had 1.5 ml (Ali and Mustafa, 1986). Patil and Raja (1978) reported ejaculate volume of 0.5 ml for Malabari goat. In general, however, ejaculate volume of PE and Boer goats in Indonesia comparable with ejaculate volume (0.1 – 1.5 ml) reported for some breeds of goat (Evans and Maxwell, 1987; Jainudeen and Hafez, 1993).

Sperms motility of PE and Boer goats in the present study was 67.14 -75.83% with live sperms of 74.69 - 82.48%. These values were slightly higher than the results of 56-66% for motility and 63 - 71% for live sperms of PE goats under village conditions (Setiadi *et al.*, 2001). Other studies reported sperms motility of PE goats was 74 - 78% (Suwarso, 1999; Tambing *et al.*, 2000). Some factors influenced sperm motility was number of ejaculate, age of buck, temperature, breed (Shukla *et al.*, 1992), collection technique, and post collection semen management (Evans and Maxwell, 1987). Sperm concentration obtained in the present study was 2.020 -2.044 billion/ml for PE goat and 2.080 - 2.085 billion/ml for Boer goat. This value was well within the range of normal sperm concentration of goat, 2000 - 6000 million/ml (Evans and Maxwell, 1987; Jainudeen and Hafez, 1993).

Table 2. Ejaculate characteristics of PE and Boer goats

Parameter	PE		Boer		P Value		
	EJ-1	EJ-2	EJ-1	EJ-2	B	EJ	Intr.
Volume (ml/ejaculate)	1.18	0 .24	0.74	0.65	*	ns	ns
Motility (%)	70.55	73.00	67.14	75.83	ns	ns	ns
Live sperms (%)	76.31	82.48	74.69	75.83	ns	ns	ns
Sperms conc. (million/ml)	2.044	2.020	2.085	2.080	ns	ns	ns
Abnormality (%)	6.53	6.87	7.11	6.96	ns	ns	ns

EJ = Ejaculate, B = Breed

Sperm concentration, volume and motility will determine dilution rate, and this will related to number of does can be inseminated. Motility may be used as an indicator of the ability of sperms to travel in the female reproductive tract and finally fertilize ovum. Similarly, abnormality is a good parameter to evaluate quality of ejaculate. The average sperms abnormality in the present study was 6.53 - 7.11% that was within normal range of 6 - 10% reported in some studies (Delgadillo *et al.*, 1992; Tambing *et al.*, 2000; Kostaman *et al.*, 2000; Sutama *et al.*, 2001). In the artificial insemination, percentage of sperm abnormality must not more than 15% (Evans and Maxwell, 1987).

Changes in Motility and Live Sperms during Equilibration and Freezing

The averages sperm motility and live sperm of Boer and PE goats during equilibration and freezing were shown in Table 3. There were no significant interactions between breed of goat and equilibration time for any parameter measured. At dilution, average sperm motility of Boer and PE goats was 69,09% and 70.62%, respectively. There was a clear decrease in sperm motility and live sperm in the first 2 hours equilibration; thereafter it seems to be constant. Breed and duration of equilibration time did not significantly affect both sperm motility and live sperm before freezing. After freezing, however, equilibration time (1-3 hours) significantly affected the quality of frozen semen obtained, in which 3-hours equilibration times gave the best results.

Table 3. The effect of equilibration time on frozen semen quality of PE and Boer goats

Parameters	PE			Boer			P Value		
	EQ-1	EQ-2	EQ-3	EQ-1	EQ-2	EQ-3	B	EQ	Intr
Motility (%)									
- Dilution	——	70.62	——	——	69.90	——			
- Equilibration	68.71	65.62	66.00	68.75	65.83	66.66	ns	ns	ns
- Post-Thawing	25.71	27.50	34.64	33.75	31.66	40.71	*	*	ns
Live sperm									
- Dilution	——	75.62	——	——	74.18	——			
- Equilibration	73.72	72.57	72.65	75.90	70.98	71.30	ns	ns	ns
- Post-Thawing	35.80	37.40	44.12	42.91	35.14	52.96	*	*	ns

EQ = Equilibration Time.

The present study showed that the larger decrease in sperm motility (39-63%) and live sperm (26 -51%) occurred during freezing, and the lowest changes occurred in 3-hour equilibration (Table 3). These figures were higher than those reported previously, 26% for motility, 15 - 18% for live sperms (Sutama *et al.*, 2001). Motility is a common parameter used to evaluate frozen semen. In the present study average sperm motility of PE goat after thawing was lower ($P<0.05$) than that of Boer goats (34.6 vs. 40.7%). Improvement of goat semen preservation technique is needed in order to get minimum sperm motility (40%) required for AI (Evans and Maxwell, 1987).

In-Vivo Test of Frozen Semen

Pregnancy rate following AI using frozen semen of PE and Boer were 31.43% and 31.25%, respectively. These results were lower than that reported previously for PE goats with once insemination (36.84 - 38.89%) and twice insemination (47.36 - 55.56%) (Sutama *et al.*, 2001). This low pregnancy rate in the present study could be due to low number of motil sperm inseminated (about 60 million/dose) shown by sperm motility of 39 - 40% (Table 4). Feradis (1999) found higher pregnancy rate (61.1%) if the ewes were inseminated with 200 million sperms/dose compared 25% if inseminated with 100 million sperms/dose. In general, most of the studies showed that pregnancy rates in goat and sheep varied and relatively low (30 - 60%) (Salamon, 1971; Samouilidins and Hahn, 1973; Visser and Salamon, 1974; Sutama *et al.*, 2001). Difficulty to penetrate cervix during insemination in goat could contribute to the low pregnancy rate obtained.

Table 4. Pregnancy rates of PE does inseminated with PE and Boer frozen semen.

Parameter	Frozen semen		
	PE	Boer	Total
Semen quality:			
- Motility (%)	40.47 ± 3.12	39.29 ± 2.06	
- Live sperm (%)	55.21 ± 4.68	52.26 ± 7.19	
Pregnancy rate:			
- No. of does inseminated (heads)	35	16	51
- No. of does pregnant, heads (%)	11 (31.43)	5 (31.25)	16 (31.37)

Conclusion

From the results of the present study, it can be concluded that 3-hours equilibration could gave a better frozen semen quality compared with 1 and 2 hours, though *in-vivo* test showed a relatively low pregnancy rate (31.37%).

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