Proportion and Quality of X-Y Chromosome Bearing Sperm on Diluted Semen after Incubation in Different Time of Etawah Crossbreed Goat

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ABSTRACT

Sperm sexing technology is one of the alternatives to predict the sex according to the wishes of farmers, in order to improve reproductive efficiency and increase the efficiency of livestock business. There is one important factors affecting in the successfull of sperm separation especially using BSA method, that is incubation time. This research was aimed to 1) determine the effect of incubation time on the proportion and quality of X-Y chromosome bearing sperm from diluted semen of Etawah Crossbreed; 2) to get the incubation time that produce the highest proportion of X cromosom bearing sperm with highest semen quality. The research use completely randomized design (CRD) with three treatments (T1 = 45 incubation time, T2 = 60 incubation time, and T3 = 75 incubation time) with 10 replications. Parameter measured were proportion X-Y sperm, motility and Intact Plasma Membrane (IPM). Data were analyzed using analysis variance followed by Duncan's multiple range test. The results showed that the largest percentage proportion of X-Y spermatozoa in the upper fraction belongs to T1 (75.40±3.20%) followed by T2 (64.40±4.58%) and T3 (53.60±2.80%). The largest percentage of bottom fraction is at T3 (81.00±2.58%) follow by T1 (67.50±5.68%) and T2 (65.00±4.47%). The results showed that the largest percentage motility of X-Y spermatozoa in the upper fraction belongs to T1 (72.89±2.13%) followed by T2 (70.57±3.82%) and T3 (68.26±3.69%). The results showed that the largest percentage IPM of X-Y spermatozoa in the upper fraction belongs to T1 (74.05±1.86%) followed by T2 (71.75±1.46%) and T3 (67.85±2.14%). Based on the results it is concluded that incubation time affect on proportion of X-Y sperm and quality of dilute semen from of Etawah Crossbreed Goats, the incubation time of 45 minutes is the optimum time to produce highes proportion of X-Y chromosome bearing sperm and diluted semen quality of Etawah Crossbreed goats.

Keywords: Sexing sperm, Incubation time, Etawah crossbreed goat

INTRODUCTION

Sexing of sperm is one of biotechnology to improve population of a particular sex of a certain animal by increase the ratio of X or Y chromosome bearing sperm. The effort to change the ratio of X-Y spermatozoa by the use of Bovine Serume Albumin (BSA) affected by several factors, one of which is the duration time to spermatozoa penetrating the BSA solution, that is called incubation time.

In order to Etawah Crossbreed goat, optimal incubation time was expected to produce highest ratio of X chromosome bearing sperm to increase the pregnancy and parturition of female goat. The regulation of the sex may suppress the acquisition of livestock from the less needed sex, thus if the goat semen which has been separated by sex is used for Artificial Insemination (AI), therefore can reach increasing of reproductive efficiency. Nevertheless, for breeding with AI, need standar quality of semen to be used. Therefore the optimal incubation time was needed to get the the highest proportion and quality of X chromosome bearing sperm in dilute semen of Etawah crossbreed goat.

The aimed of this research were to 1) determine the effect of incubation time on the proportion and quality of X chromosome bearing sperm from diluted semen of Etawah Crossbreed; 2) to get the incubation time that produces highest proportion of X cromosom bearing sperm with highest semen quality.

MATERIALS AND METHODS

The object of this research was semen from Etawah crossbreed goat, two years in old. Semen was collected with an artificial vagina (AV) for 10 times as replication. Immediately after collection, semen was evaluated as macroskopik and microskopik evaluation.

We use Bovine Serum Albumin (BSA) as much two fractions (10% in bottom fraction and 5% in upper fraction) as sexing media. Bovine Serum Albumin was diluted in BO (Brackett–Oliphant) medium and placed in the tube as much 2 cm in high for each fraction. The semen was diluted in BO medium and placed in the tube as much 1 cm in high.

The treatment consists of three incubation time: T1 (45 minutes), T2 (60 minute) and T3 (75 minutes). Each treatment were repeat for six times. The parameter consists of: the proportion of X chromosome bearing sperm and semen quality include of motility, intact plasma membrane (IPM). Determination of X chromosome bearing sperm based on morfometrik evaluation use aplication DP2-BSW with magnification 10x100. The number of spermatozoa calculated from each fraction is 200 cells, whose head size is greater than the average is categorized as X chromosome bearing sperm (X sperm), whereas if the head size is smaller than the average it is categorized as the Y chromosome bearing sperm (Y sperm). Evaluation of motility using Neubauer counter, and for evaluation of intact plasma membrane using hypoosmotic swelling (HOS) tes (Jayendran and Zenevald, 1986). Data was analized with analisys variance and the differences between treatment was analized with Duncan test.

RESULTS AND DISCUSSION

Characteristic of fresh semen

Result of macroscopic and mikroscopic evaluation of fresh semen showed that the semen have normal characteristic. The volume is 0.63 ml/ejakulat in average. According to Arifiantini (2012) and Tambing *et al* (2000), an average of Etawah Crossbreed goat is 0.5-2.00 ml and 1.08 ± 0.47 ml respectively. The color is creamy. Consistency is rather dilute. The odour is a tipically. PH is 6,93 in average. The mass movement is +++. Total sperm concentration is $3.05x10^9$ cells/ml in average. Motility is 85,36% in average. According to Suwarso (1999) motility of Etawah Crossbreed goat was 78,13%. The quality of semen obtained is good enough, so it is possible to pass further process. According to Affandhy *et al*. (2004), standar quality of semen were motility >70% sperm concentration >750 juta/ml ejaculate with consistensy medium to thick and white in colour until creamy.

Effect of incubation time on proportion of x chromosome bearing sperm

Result of ANOVA showed that incubation time significantly (P<0,05) effect on proportion of X chromosome bearing sperm. Result of Duncan test showed that motility of each treatment had significantly different. This result means that incubation time for 45 minutes is the optimum time to get the highest proportion of X chromosome bearing sperm in Etawah Crossbreed goat. This finding on proportion of X chromosome bearing sperm higher than proportion was reported by Hendri (1992) on goat sperm use BSA 6% for 6 ml as much 61.00% and Putra *et al.* (2012) that reported got X chromosome bearing sperm as much 71,50% and 72,30% with incubation time 10 and 20 minute respectively. Nevertheless, Afiati (2004) reported that bull semen incubated for 60 minutes using egg albumin resulting proportion of X chromosome bearing sperm as much 80.88%

Tabel 1. Result of duncan test on proportion of x chromosome bearing sperm

Treatment	Proportion of X Sperm(%)	Significancy (P<0,05)
T1 (45 minute)	75.40±3.20	A
T2 (60 minute)	64.40±4.58	B
T3 (75 minute)	53.60±2.80	C

Different alphabet in same colums mean significant different

Effect of incubation time on motility

Result of ANOVA showed that incubation time had significant (P<0,05) effct on motility. Result of Duncant test showed that motility T1 significanlty different wit T2 and T3,

also T2 significantly different with T3. According to Saili (1999) decrease of motility occure because of the sperm had passed tretment and need much energy to maintenace the normal fisiological condition. Semen washing process cause decrease of plasma semen concentration. Situmorang *et al*. (2013) also report that motiity after separation for 30 minutes (74.4%) lower than separation for 20 minute (77.5%) and 10 minutes (79.4%). Therefore, this showed that addition the time for incubation during sperm would increase sperm metabolism and thus could decrease semen quality.

Afiati (2014) report that motility with 60 minute incubation time is 70,83%. Motility from our research higher than motility was reported by Saili (1999) in Ongol Crossbreed cow with colom albumin, that is 64%.

Table 2. Result of duncan test on motility

Treatment	Motility (%)	Significancy (P<0,05)
T1 (45 minute)	75.89±2.13	A
T2 (60 minutes)	70.57±3.82	Ab
T3 (75 minutes)	68.26±3.69	B

Different alphabet in same colums mean significant different

Effect of incubation time on intact plasma membrane (ipm)

Resulf of ANOVA showed that incubation time significantly ($p<0.05$) effect on IPM. Result of Duncan test showed that every treatment showed significantly ($p<0.05$) different each other. The value of IPM from this reseach higher than the result of Afiati (2004) as much 62,04%. The decrease of IPM was caused by treatment during the sexing process that caused sperm loss of ability to mainenance intracellular liquid. Sperm sexing process with centrifugation caused fosfolipid. Sexing by centrifugation process can lead to loose partially of the fosfolopid membrane of spermatozoa resulting from mechanical influences, namely the centrifugal force. The partial loss of membrane phospholipids may cause the integrity of the membrane to be impaired, thus affecting the viability of the membrane. According to Diliyana *et al*. (2014), good membrane integrity of spermatozoa was demonstrated that phospholipids can survive and keep well to the collision between the tube and the medium when sexing.

Table 3. Result of duncan test on intact plasma membrane (ipm)

Treatment	IPM (%)	Significancy (P<0,05)
T1 (45 minute)	74.05±1.86	A
T2 (60 minute)	71.75±1.46	B
T3 (75 minute)	67.85±2.14	C

Different alphabet in same colums mean significant different

Table 4. Proportion of x chromosome bearing sperm and dilute semen quality after treatment of incubation time

	T1 (45 minute)	T2 (60 minute)	T3 (75 minute)
Proportion X sperm (%)	75.40±3.20	64.40±4.58	53.60±2.80
Motility (%)	75.89±2.13	70.57±3.82	68.26±3.69
Intact Plasma Membrane (%)	74.05±1.86	71.75±1.46	67.85±2.14

CONCLUSIONS

The proportion of X chromosome bearing sperm and diluted semen quality was affected by incubation time, therefore optimal incubation time that produces highest proportion of X chromosom bearing sperm and diluted semen quality is 45 minute.

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