International Asia Link Symposium: "Reproductive Biotechnology for Improved Animal Breeding in Southeast Asia"

Biology of reproduction and artificial insemination of Timor deer (Cervus timorensis)


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Timor deer (Cervus timorensis) is the native tropical deer in Indonesia and is one of 20 wild animals which have been domesticated fairly recently for meat production (Kyle, 1994). As a newly domesticated farm animal which is highly potential and prospective as a source of animal protein, efforts should be taken into account to increase its population and productivity through the application of appropriate reproductive technology, particularly artificial insemination (AI). Prerequisite for the application of AI technology in timor deer is an intensive study of its biology of reproduction.

A series of research activities have been conducted to study the biology of reproduction and AI of the timor deer, encompassing the pattern of antler developmental cycle in relation to testosterone hormone profile and sperm production of the stag, anatomy of reproductive organs of the stag and hind, semen characteristics, preservation, and cryopreservation of the timor stag semen. Estrous cycle and profile of the hind steroid hormones, estrous synchronization and insemination of timor hinds using frozen-thawed semen of the stags.

Starting from the pedicles on the dorsolateral sides of the head of the stag, the bony antler developed from the velvet stage to the hard antler and casting stages, which lasted for about 148.8, 208.8 and 16 days respectively during the whole antler developmental cycle. These cyclic stages of the antler development correlated with and determined reproductive activities of the adult timor stag, particularly with testosterone hormone and sperm production. Significantly higher semen concentration of the stag, anatomy of reproductive organs of the stag and hind, semen characteristics. preservation, and cryopreservation of the timor stag semen, estrous cycle and profile of the hind steroid hormones, estrous synchronization and insemination of timor hinds using frozen-thawed semen of the stags.

The reproductive organs of timor stag resemble those of the small ruminant, except that it has no flexura sigmoidea at the caudal part of the penis. The testis has a diameter of 36.55 mm and weighs about 108.11 g and the total length of the penis is 43.75 cm with the diameter of about 2.1 mm. Testes of the timor stag is relatively smaller than that of the ram. Semen characteristics of timor stag should only be evaluated at the hard antler stage. The cream-coloured semen has a volume of about 1.5 ml resembling that of the ram (Rizal, 2004), with higher pH (about 7.7) compared to cattle (6.8) (Toelihere, 1993) and sheep (7.0) (Rizal, 2004). Sperm concentration of about 840 to 1.4 million ml⁻¹ mostly resembles that of the bull than the ram (Garner and Hafez, 2000). Sperm motility during the hard antler stage of about 70% does not differ from that of the bull (Garner and Hafez, 2000) as well as of the ram (Rizal, 2004). The stag semen is well pre-
served in Tris glucose and Tris succrose extenders; its motility in liquid semen stored at room temperature (27°C) reached around 40% 15 hours after dilution and reached more than 42% after 9 days in the refrigerator (3-5°C). The best glycerol concentration for storage of semen cryopreservation in Tris succrose extender is 10% with the after thawing motility reached more than 50%, far above the minimum requirement for frozen-thawed mammal semen (Hafez, 2000; Toelihere, 2004a).

Generally the reproductive organs of the timer hinds resemble those of ewes and does. The left ovary is heavier (about 0.94 g) than the right one (about 0.88 g) which is slightly bigger than the ovary of heifers (Mordhiana, 2001). The uterus horns in hind are longer (about 12 cm) than that of wapiti deer (5.8 cm) (Haigh et al., 1993) but resembles that of the ewe (10 to 12 cm) (Hafez, 2000). The cervix is relatively short (about 5 cm) and has 4 to 5 annular rings compared to only 3 to 4 in cows (Hafez, 2000). After analysis on steroid hormones of the hind, it is obvious that the lowest progesterone level (0.2 Î¼g ml⁻¹) coincided with the highest level (18.14 Î¼g ml⁻¹) of estrogen which was sequentially repeated at an interval of 17 days which determined the length of estrous cycle in the hind. This is the typical length of estrous cycle in the female tropical deer with a range of 10 to 25 days, the shortest (10 to 18 days) being in the timer deer and the longest (12 to 23 days) in the spotted deer (Semiadi, 1995). The estrous period itself lasted for about 24 hours. The estrous period may last longer than 24 hours (Guiness et al., 1971). On the contrary Fennessy et al. (1988) reported that estrous signs in the female deer lasted only 12 to 14 hours. Estrus as the result of PGF₂α treatment in bawean deer lasted for more than 24 hours. Semiadi (1995) concluded that the estrous period in tropical deer is shorter than in temperate deer. A hind is detected to be in heat when she accepted a stag to be on her side, which shows aggressive behavior and fight other stag in the pursuit of containing and protecting the hind as its own possession. The hind in estrous will sniff the perinae area of other hinds and may mount each other. The vulva becomes swollen and there is a distinct and clear vaginal discharge of the hind in heat, which moves its buttock and feet forward and backward. The hind in heat will produce specific sounds, it is uneasy but stand still when its back is pushed down and yields if its vulva is touched by the keeper. Natural mating takes place three to four times within two hours of the estrous period.

Estrous synchronization using CIDR-G to control estrous cycle in 12 to 18 cows was conducted with good results of 42%. The pregnancy rate reached 60 to 100% at the second and third trial without anesthesia. The gestation length reached 248 to 277 days. It is generally concluded that reproductive activities of the timer stag coincide with the hard stage of the antler development cycle. no fixed ligament is at the distal part of the penis and less centraled sperm cells in ejaculate of the stag compared to that of the of the timer hind resemble those of the ewe with the estrous cycle. Estrous symptoms last for about 24 hours. It readily responds to CIDR in estrous synchronization with good results of 81% under no and semen after cryopreservation with Tris sucrose extender containing gel. The pregnancy rate reached 60 to 100% and the length of gestation.

References


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contrasted sperm cells in ejaculate of the stag compared to that of the ram. Reproductive activities of the timer hind resemble those of the ewe with the estrous cycle length of about 17 days and estrous symptoms last for about 24 hours. It readily responds to CIDR-G intravaginal implantation in estrous synchronization with good results of AI under no anesthesia using frozen thawed semen after cryopreservation with Tris succrose extender containing eight to ten percent of glycerol. The pregnancy rate reached 60 to 100% and the length of gestation reached 248 to 277 days.

References
Quality of frozen-thawed Holstein bull sperm following centrifugation and Percoll gradient treatments.

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Hafiez (2001) reported that X and Y sperm have different characteristics and can be separated. Susilawati et al. (2001, 2003a, b) have successfully separated sperm sexing using centrifugation and the result showed proportion of 91% of total sperm count fractionated to X and Y sperm, respectively. The X and Y sperm proportion x 100 was calculated for each fraction of centrifugation and Percoll gradient treatments. In our research, X and Y sperm were separated using centrifugation and Percoll gradient treatments. In addition, we compared the results of centrifugation and Percoll gradient treatments with artificial insemination (AI) and evaluated the fertility of semen samples. The overall results showed that the fertility of semen samples was higher than 50% for both treatments. However, the fertility of semen samples was significantly different between treatments, with the centrifugation method resulting in higher fertility than the Percoll gradient method for both X and Y sperm.

The objective of this research was to optimize the quality and proportion of sperm used for AI by comparing two different treatments: centrifugation and Percoll gradient method. The research was conducted at Singosari AI centre, Malang, East Java, Indonesia. All semen samples were collected from the Holstein bulls and separated into three groups: 1, 2, and 3 ml of semen volume for separation process. The semen was separated using centrifugation and Percoll gradient treatments. The semen samples were tested for motility, viability, and abnormality. The results showed that the centrifugation method resulted in higher semen quality than the Percoll gradient method. The proportion of X sperm was significantly higher in the centrifugation method than in the Percoll gradient method.

Table 1. The Y sperm quality on top fraction after centrifugation and Percoll gradient treatments

<table>
<thead>
<tr>
<th>Treatments</th>
<th>1 ml</th>
<th>2 ml</th>
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<tbody>
<tr>
<td>Motility (%)</td>
<td>57±4.5</td>
<td>59±5.7</td>
</tr>
<tr>
<td>Concentration (million/ml)</td>
<td>117±6.7</td>
<td>116±6.9</td>
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<tr>
<td>Viability (%)</td>
<td>86±2.3</td>
<td>83±2.8</td>
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<tr>
<td>Abnormality (%)</td>
<td>3±1.0</td>
<td>3±1.2</td>
</tr>
<tr>
<td>Proportion of Y sperm</td>
<td>87±2.8</td>
<td>89±2.8</td>
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</tbody>
</table>

Increasing semen volume used for separation has affected the proportion of Y sperm, but not affected the motility, viability, abnormality, and proportion of Y sperm significantly.