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 (Al). Prerequisite for the application of Al 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 tirnor 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 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 stags, particularly with testosterone hormone and sperm production. Significantly higher semen quality and higher testosterone hormone level were obtained during the hard antler stage (sperm motility 70.46%, sperm concentration 978.25 million ml^{-1}, abnormal sperm 9.64%, and testosterone hormone level 16.71 $\mu g\ ml^{-1}$) compared with the velvet stage (sperm motility, sperm concentration and sperm abnormality as well as testosterone hormone level were 32.74%, 208.97 million ml^{-1}, 50.87% and 0.73 $\mu g\ ml^{-1}$ respectively).

The reproductive otgans of timor stag resemble those of the small ruminant, except that it has no *flexura sigmoides* 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 21 mm. Testis of the timor stag is relatively smaller than that of the ram. Semen characteristics of timor stags 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 (Gamer and Hafez, 2000) as well as of the ram (Rizal, 2004). The stag semen is well pre-

served in Tris glucose and Tris sucrose extenders; its motility in liquid semen stored at room temperature (27-28°C) reached around 40% 15 hours after dilution and reached more than 42% after 9 days in the refreegerator (3-5°C). The best glycerol concentration for stag semen cryopreservation in Tris sucrose extender is 10% with the after thawing motility reached more than 50%, far above the minimum requirement for frozen-thawed mammalian semen (Hafez, 2000; Toelihere, 2004).

Generally the reproductive organs of timor hinds resemble those of ewes and does. The left ovary is heavier (about 0.94 g) than the right one (about 0.68 g) which is slightly bigger than the ovary of ewes (Mardhiana, 2001). The uterine horn of timor hind is 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 5cm) 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 μg ml $^{-1}$) coincided with the highest level (t 8.14 pg 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 timor 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 (1985) reported that estrous signs in the female deer lasted only 12 to 14 hours. Estrus as the result of $PGF_2\alpha$ 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 stags in the pursue of containing and protecting the hind as its own possession. The hind in estrous will sniff the perineal 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 the hinds has been practiced by many research workers (Asher, 1989; Mylreal, 1992). CIDR-G was implanted intravaginally for 12 to 14 days and the hinds would come in heat 48 hours after release of CIDR-G. The same results were obtained in the study of estrous synchronization in timor deer. All six hinds after repeated treatments under intravaginal CIDR-G implant for 14 days came in heat 25 hours after the release of CIDR-G and estrous symptoms lasted for about 28 hours. The same results were also obtained by other workers in sheep (Feradis, 1999; Ngangi, 2002).

As small ruminant, a speculum should be used in the technique of insemination in the hind. AI under anesthesia gave poor results. AI should be carried out without anesthesia, but the hind should be put in a restricted confinement made up of a wooden crate which keeps the hind tight in place. Anesthesia would cause uterine relaxation which hampered sperm transportation toward the location of fertilization in the fallopian tube. AI was conducted at the end of estrous period with frozen thawed stag semen in 0.25 ministraw containing about 150 million sperm and deposited at the first cervical annular ring. The AI resulted in a pregnancy rate of 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 timor stag coincide with the hard stage of the antler development cycle. no *flexura sigmoides* at the distal part of the penis and less con-

centrated sperm cells in ejaculate of the stag compared to that of the ram. Reproductive activities of the timor 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 sucrose 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.

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