Use of Pregnancy-Associated Glycoproteins (PAG) Levels to Determine Early Pregnancy in Somatic Cell Clone Recipient Buffaloes

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Introduction
The swamp buffalo is an important livestock in Thailand. It is well known that buffalo has low reproductive efficiency which is one of the cause that the population of buffalo has been gradually declined. Cloning is a technique for selection and conservation of the valuable Thai swamp buffalo. Somatic cell nuclear transfer (SCNT) is a cloning technique has been used to reproducing the superior livestock or to preserve rare or endangered species. The SCNT animal has been reported in cattle, swine, sheep, and goats. Successful cloning of swamp buffalo by SCNT has been already reported (10,11,13). However, cloned embryos produced by somatic cell nuclear transfer technique resulted in a low percentage of successful pregnancy. Early embryonic loss due to defect in the cloned embryos or failure to implant in the uterus of the recipient have been previously reported (4). Detection of early pregnancy status may improve the successful pregnancy.

Pregnancy-associated glycoproteins (PAG) belong to a family of aspartic proteinase (16) synthesized by mono- and binucleate trophoblastic cells in ruminants (15). During pregnancy, PAG can be served as biomarkers of pregnancy, it was detected in maternal blood circulation soon after implantation and throughout the gestation in ruminant including buffalo (17).

Radioimmunoassay (RIA) and Enzyme-linked immuno-sorbent assay (ELISA) using monoclonal or polyclonal antibodies have been developed to measure PAG concentration. Determination of PAG for pregnancy diagnosis was highly accurate and was correlated with the time of gestation (7). In buffalo, the sensitivity of PAG-RIA test was 11.1% at Day 19–24 and reached 100% from Day 31 after mating (7). The specificity of PAG-RIA test ranged from 90 to 100% from Day 19 to 55 after mating (6). A sandwich ELISA, using anti-PAG monoclonal antibodies, which were able to detect PAG in all pregnant animals on day 28 (2). Moreover, PAG had significantly higher specificity than progesterone for diagnosis of non-pregnant ewes (5). The molecular structure of PAG are closely related among the different species in ruminants thus PAG concentrations can be determined by using antiseras raised against PAG from the other species. Differences of PAG concentrations were depended on antiseras and condition of the assay systems (1). Recently, commercial ELISA kits for PAG have been produced for pregnancy diagnosis. It has been developed for determination of pregnancy in bovine and was also used to determine pregnancy status in buffalo with high sensitivity and specificity (8). PAG have been used as a new technology for early pregnancy diagnosis after artificial insemination (AI), however, there is no information of PAG levels in somatic cell clone recipient buffalo. The aim of the study is to determine whether PAG can be used for early pregnancy diagnosis in somatic cell clone recipient buffalo.

Materials and Methods
Twelve cycling swamp buffaloes possessing a regular oestrous cycle by their respective plasma progesterone profiles were selected as somatic cell clone recipients. Day 6 SCNT embryos were produced as previously described (3). One to three embryos were transferred into the recipient buffalo at day 6 after detection of oestrous. Following embryo transfer, blood samples were collected from the jugular vein at 10-days intervals and then collected to Day 150. Blood samples were centrifuged at 1500 g for 15 min and the plasma was stored at −20 °C for PAG analysis. Pregnancy status was diagnosed by transrectal palpation at Day 60.

Plasma PAG concentrations were determined with a commercial ELISA test (IDEXX bovine pregnancy test, IDEXX Laboratories, Switzerland, AG). The sample and control were tested according to the manufacturer assay protocol. The results were measured the optical density at 450 nm using a microplate reader (EL311 BioTek, USA). The PAG levels of the samples were determined by calculating from the optical density (OD) at 450 nm of the sample (S) minus the OD of negative control (N); (S- N value). The cut off value recommended by the manufacturer, if PAG value was equal or greater than 0.300, the recipient was considered pregnant; if PAG value was less than 0.300, the recipient was considered non-pregnant. The inter-assay and intra-assay coefficients of variation of quality control concentrations were 6.1% and 3.7%, respectively.

249
Results and Discussion

As measured by IDEXX, all of the somatic cell clone recipient buffaloes had similar PAG concentrations which below the cut off value until day 20 after embryo transfer as shown in Figure 1.

![Figure 1](image)

Figure 1 PAG profiles of 1 pregnant and 11 non-pregnant somatic cell clone recipient buffaloes by a commercial ELISA test. The black arrow indicated the point at which the PAG levels of the pregnant somatic cell clone recipient buffalo became detectable and reached above the cut off value.

In one of the recipient buffaloes, PAG concentrations became detectable and reached above the cut off value on Day 30 and maintained at the high levels through Day 150. The PAG concentrations showed that only one of the recipient buffaloes might be diagnosed as pregnancy. The pregnancy status was confirmed by transrectal palpation on day 60, result showed that there were 1 pregnant and 11 non-pregnant recipient buffaloes. The result of pregnancy by transrectal palpation was agreed with the PAG concentrations. The pregnant recipient buffalo maintained pregnancy to term at 326 days of gestation.

The PAG profile of the pregnant recipient buffalo in the study is resembled to the previous studies in normal AI buffalo (8) and goat (14) which resulted that PAG levels were increased at the early stage of pregnancy and maintained at the high level throughout the gestation period. The PAG profiles not only for early pregnancy diagnosis but also it can be used for determined early embryonic mortality (12). Decreased serum concentrations of PAG significantly increased the probability of late embryonic mortality in lactating dairy cows (9).

Interestingly, in non-pregnant recipient buffaloes, the PAG concentrations were below the cut off value which may be associated with pregnancy failure from either an abnormal and/or poorly developed placenta (3).

In conclusion, the study demonstrated that determination the PAG levels in somatic cell clone recipient buffaloes by the commercial ELISA test can be applied for early pregnancy diagnosis on Day 30 after embryo transfer onwards.

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References