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Expression of cumulus-oocyte complex genes and embryonic development in goats subjected to progestogen-based estrus synchronization

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ABSTRACT

The objective was to investigate the effect of short-term (7 days) and long-term (14 days) progesterone-based estrus synchronization on number of follicles, progesterone concentrations, cumulus-oocyte complex (COC) gene expression, and embryonic development in goats. Nulliparous Thai-native goats (n = 45) were randomly assigned to one of two estrus synchronization treatments. Goats were treated with intravaginal sponges containing 60-mg medroxyprogesterone acetate (MAP; Synchrogest esponjas, Spain) during 7 or 14 days (short-term or long-term protocol, respectively). Multiple follicular development was induced by intramuscularly injections of 300-IU eCG in both groups (1 day before sponge withdrawal). An ovariectomy was performed at 24 hours after sponge removal to evaluate number of follicle and collect oocyte for IVF. Oocyte quality (healthy or non-healthy) was determined by morphology of COCs before IVM. Recovery of COCs and total cellular RNA isolation were applied to determine apoptosis-related gene expression. After IVF, embryos were evaluated during the eight-day culture as numbers of cleaved oocyte, morula, and blastocyst embryo. Total numbers of follicles and oocytes were similar for both treatments. Plasma progesterone concentrations were not different during MAP insertion period ($P > 0.05$). However, goats that received the short-term protocol had a greater number of 4 to 6-mm follicle, healthy oocytes, cleaved oocytes, and morula embryos than goats that received the long-term protocol ($P < 0.01$). In addition, the expression of B-cell lymphoma 2 messenger RNA was greater ($P < 0.05$) in COCs derived from the 7 days MAP-treated when compared to the 14 days MAP-treated goats. These data highlight that the 7-day progestin–based treatment may contribute to quality of oocytes and embryonic development in goats.

1. Introduction

Progestins have been widely used in sheep and goats to induce and synchronize estrus [1,2]. Whereas gonadotropins (FSH and eCG) have often been used to stimulate ovarian activity after progestin treatment and have provided a more compact ovulation in sheep [3] and goats [4,5]. The most commonly used estrus synchronization protocols in goats include medroxyprogesterone acetate (MAP) and fluorogestone acetate vaginal sponges. For successful estrus synchronization, (100% in estrus within 4 days) 60-mg MAP sponges were applied for 14 days with or without 500 IU of eCG at sponge removal [6]. However, long-term use of MAP treatment has been associated with

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lower fertility in cows [7,8], sheep [9], and goats [6,10]. Reduced fertility in prolonged progestin-treated animals may also be related to an impairment of sperm transport in the genital tract and survival reducing the number of fertilized ova [11].

To overcome these problems, progestin administration can be shortened to 5 to 7 days in combination with injection of $PGF_{2\alpha}$ or gonadotropins at the time of withdrawal in sheep [12] and goats [13–15]. In addition, the short-term progestogen treatment reduces persistent ovarian follicles [14]. Higher pregnancy rates were observed after a short-term progestin treatment (6 days of MAP) compared to the traditional treatment [13,16]. The use of progestins in combination with eCG at withdrawal results in a highly variable response in goats [17,18] and reduces both recovery rates and yield of transferable embryos [19]. However, in goats, progestins have been found to have no effects [20,21] or depressed fertility and kidding performance [22,23]. In Thai-native goats, estrus and ovulatory response were not different between 14-day and 7-day treatments with eCG [24]. Recently, once-used synthetic progestogen with gonadotropin has been used for estrous synchronization in native goats without negative effect [25].

Quality of oocyte has a critical role in embryonic development after fertilization, and thus can be determined by its ability to be fertilized and develop into a healthy embryo or offspring or by expression of specific gene markers [26,27]. Extensive research on IVM, IVF, and IVC of the resulting zygotes has so far been reported [28,29], but limited information has been found on the evaluation of goat ovaries and the efficiency of oocyte collection, and evaluation [26]. Although the benefits associated with estrus synchronization in goats have been reported, specific studies focused on cumulus-oocyte complex (COC) genes expression and embryonic development after estrus synchronization and superovulation have not been evaluated in native goats. Therefore, the present study was undertaken to evaluate the effect of 7-days and 14-days progesterone–based estrus synchronization on number of follicles, progesterone concentrations, COC gene expression, and embryonic development in native goats.

2. Materials and methods

2.1. Animals and welfare

This experiment was carried out at the small ruminant unit, Department of Animal Science, Faculty of Agriculture, Khon Kaen University, located at 16° 26′ N latitude and 102° 50′ E longitude, Thailand. The average annual temperature and precipitation in this area was 26.4 °C and 1459 mm^3. All experimental procedures were approved by the animal ethic committee of Khon Kaen University. Native goats used in this study were characterized as brown or black in color with a black strip along the back and small in size with short upright ears. The average weight of mature female goats was 25.3 ± 1.10 kg [30,31]. Nulliparous (n = 45) goats, aged 10 to 11 months with a body weight between 23 and 25 kg were fed a maintenance diet with ad libitum feeding of fresh ruzi grass with free access to clean water and mineral supplement as previously described [24]. Body condition score was evaluated once a week with the use of a 5-point scale on the basis of external fat cover on the spinal and transverse process at the 13th rib. Before the study, animals were dewormed and vaccinated against foot and mouth disease, hemorrhagic septicemia, and brucellosis according to the standard farm requirement of the Department of Livestock Development, Ministry of Agriculture and Cooperatives, Thailand.

2.2. Estrus synchronization and superovulation

Experimental goats were randomly assigned to one of two treatment groups. Goats in group 1 were synchronized using the short-term protocol (7 days MAP and injection of PMSG) by which animals were inserted with intravaginal sponges containing 60-mg MAP (Synchrogest esponjas, Spain) for 7 days and were intramuscularly injected with 300-IU PMSG (Synchrogest PMSG, Spain) on Day 6 (1 day before sponge withdrawal). Goats in group 2 were inserted with the same progestin for 14 days (the long-term protocol) and were intramuscularly injected with 300-IU PMSG on Day 13 (1 day before sponge removal).

2.3. Oocyte collection

Goats were injected with 0.075-mg xylazine (Rompun; L.B.S. Laborator, Thailand) and 100-mg ketamine hydrochloride (Ketaset; Wyeth Animal Health, Canada), and an ovariectomy was performed to determine the number of follicles at 24 hours after sponge removal as previously described for ewes [3] and goats [24]. Ovaries were collected and swiftly transported to the laboratory. All visible follicles were then classified by diameter into large (≥7 mm), medium (4–6 mm), or small (≤3 mm) as described [26].

2.4. In vitro oocyte maturation and fertilization

Cumulus-oocyte complexes were isolated by cutting each visible follicle with a scalpel blade and flushing it two times with oocyte collection medium supplemented with heparin (TCM-199; Sigma, St. Louis, MO, USA). Under a stereomicroscope, COCs were recovered and transferred to a petri dish that contained collection media without heparin. Then, COCs were evaluated on the basis of morphology and categorized as healthy and nonhealthy as previously described [26,27]. Healthy COCs from follicles were washed three times in maturation media (TCM-199 containing 10% fetal calf serum, 2-mM glutamine, 0.25-mM sodium pyruvate, and 100-μg/mL streptomycin). Cumulus-oocyte complexes from less than or equal to 2-mm follicles were not used for IVF. Cumulus cells were removed using a stripping pipette, and oocytes were incubated in maturation media for 24 hours at 39 °C in 5% CO_2 and 95% air. Only healthy oocytes were used for IVF and were transferred to fertilization media incubated overnight under mineral oil as described [28]. The swim up technique was applied to separate viable sperm from frozen buck semen. A concentration of 1.0×10^6 spermatozoa/mL was added to the IVF media containing the oocyte. They were incubated under mineral oil for 18 hours at 39 °C in 5% CO_2 and 90% N_2. Then,