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Comparison on the efficiency of estrus synchronization methods for artificial insemination in goats

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Nowadays, estrus synchronization combined with artificial insemination has routinely been used in the breeding management of our livestock to increase/improve the genetic merit of offsprings. In this study, various methods of ES in goats were evaluated for estrus induction followed by AI using frozen-thawed semen during the non-breeding summer season. Apparently healthy, normal cycling does (n=40) of either Anglo-Nubian, Saanen and/or Boer breed that had kidded at least once, with body condition score of 3 or better, weight of 24 kg or more and 1 – 3 yrs of age were used. The does were randomly assigned to either, a) HCM b) HCM + GnRH c) Ov Synch and, d) CIDR method. Locally processed frozen-thawed semen from AN or Boer buck of known fertility were used for AI (2x; AM-PM). Among treatment groups, overt manifestation of heat showed no significant differences. The pregnancy rate was evaluated 60 days post-insemination based on changes in the body conformation of the does and confirmed by using transabdominal ultrasonography (5MHz frequency). The observed pregnancy rate for HCM, HCM + GnRH, Ov Synch and CIDR methods were 30.0%, 20.0%, 0.0% and 40.0%, respectively. Overall, the ES methods used were equally efficient in terms of estrus induction resulting to pregnancies except for OV Synch method. However for long term application, HCM method appeared most practical in terms of cost-efficiency.

Keywords: estrus synchronization, artificial insemination, pregnancy, goat

Introduction

In Philippine agriculture, the total livestock contribution is less than 50.0 %. Within it, goats contribution to livestock production value is only about 2.0 %. Nonetheless, for the past two decades, goats population was almost doubled (eg., 2.2 million in 1986 to more than 4 million at present) and the demand for

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chevon production exhibited a sustained and continuing growth. Moreover, a noticeable movement towards goat commercialization with commercial farms increasing in number and accounting for 1.03 % of inventory in 2009 compared to only 0.04 % in 1994 (Ocampo, 2012). A major constraint however to goat farmers is on how to increase the production and productivity of its local herd. The government responded by implementing the Genetic Resource Improvement Program by the infusion of exotic breeds (eg., Saanen, Toggenburg, Anglo-Nubian (AN), French Alpine, Jamnapari and Boer). In addition to production of cross-breds by natural means, an alternative approach for genetic improvement through Assisted Reproductive Technologies (ART's; eg., estrus synchronization (ES) and artificial insemination (AI) have been considered.

In tropical areas, local goats are said to be capable of reproducing throughout the year, whereas season is a factor when importing different goats breed. It is in this area where ES becomes important for both technical (synchronization of kidding, adjustment to forage availability or continues meat/milk supply) and genetic reasons (improved genotype dissemination by AI or natural means). ES allows for parturition at suitable times to take advantage of niche markets, feed supplies, labor and rising price trends. Before, ES in goats has focused mostly on dairy goats for optimal timing of milk production. However, increasing demand in chevon has resulted to the use of other ruminant ES regimens in meat goat management systems. Their efficiency however, is affected by many factors, including the seasonality of goat reproduction, leading to fluctuations in the expected results and availability of final products. Among the methods of synchronization adopted have included techniques as simple as alteration of light patterns or manipulation of social inputs (Whitley and Jackson, 2004) and as complex as varying timed hormonal treatments combined with light alteration and the buck effect (Boscos et al., 2002; Pellicer-Rubio et al., 2008). ES using timed hormonal treatments appeared more convenient in many meat goat production situations. Hormones used include melatonin, progestogens (administered orally, as an injection, or by using intravaginal releasing devices), gonadotropins/GnRH (or agonists), and PG alone or in combination (Freitas et al., 2004;Whitley et al., 2004; Titi et al., 2010; Martenucci et al., 2011). As observed with sheep and cattle, breed and/or breed type, stage of production, and environmental effects can influence synchronization success in goats. The introduction of developed breeds (eg., Boer) found superior to local breeds for rapid growth and to increase the consumer and producers interest have added to the impetus for developing cost-efficient and highly effective ES regimens. In this study, various ES methods that has been attempted were applied in goats

herd during the non-breeding season and maintained in a close-confinement system with provisions for natural photoperiod. Efficiency of the methods evaluated were based on "heat" induction, as manifested by both physical, behavioral and physiological changes, resulting fecundity after AI and cost-efficiency.

Materials and methods

Source of chemicals

Lutalyse® (dinoprost tromethamine) and Controlled Intravaginal Drug Release (Eazi –Breed CIDR® Sheep and Goat Device, contains 0.3 g progesterone in inert silicone elastomer) were purchased from Pharmacia & Upjohn Co., Kalamazoo MI 49001 USA and A.C.N. 000 185 526; 59 Kirby St., Rydalmere NSW 2116, respectively; Cystorelin® (gonadorelin diacetate tetrahydrate) was supplied by Merial LLC, Duluth, GA 30096-4640, USA. All other chemicals/reagents used were obtained from Sigma Chemical Co., St. Louis, MO, USA, unless otherwise indicated.

Source of semen for AI

Locally processed frozen semen (Beltran et al., 2013) from apparently healthy bucks (eg., Anglo-Nubian, Boer), 2-3 yrs old were used in the study. Briefly, semen samples were collected early in the morning using an artificial vagina filled with warm water (42-44°C) and evaluated for volume, color, consistency, pH, percent motility and concentration. Only semen samples with 60% and above motility were selected for processing. The buffer solution composed of 320 mM (3.875 g) Tris (hydroxymethyl) amino methane, 10 mM (2.125 g) Citric acid monohydrate, 3 mM (0.625 g) Fructose and 8 mM (0.535 g) Raffinose dissolved in 100 ml triple distilled water. During extender preparation, Tris buffer solution was placed in sterile plastic test tubes, followed by egg yolk (5%) then glycerol. Before the initial dilution, the semen and the extender were allowed to warm in a water bath at 28°C for equilibration. The extender was added to the semen at 2:1 ratio for every given volume of semen. Thereafter, the tubes with extended semen were placed in a water bath pre-cooled at 15-20°C before transferring to the refrigerator (5°C) for further cooling. The remaining volume of extender was added after the initially diluted semen reached a temperature of 5-7°C inside the refrigerator. The total volume of extender to be added to the semen was based on the formula;

$$\text{Total volume of extender} = \frac{\text{Semen volume x \% Motility x Sperm concentration}}{\text{Desired sperm concentration } (10 \times 10^7/\text{ml})}$$

Cooled semen was then poured in the bubbler dish. Two to three straws were loaded with semen by sucking the semen from the bubbler dish, sealed with colored polyvinyl powder, submerged in a styrofoam box with 5°C pre-cooled water to allow complete sealing, dried and arranged in a metal tray prior to freezing. Accordingly, the tray with semen straws was suspended for 7 min above LN_2, keeping a gap of about 4 cm between the surface of LN_2 and the semen straws, before plunging directly into the LN_2, placed in a goblets and stored at -196°C until use.

Estrus synchronization methods

Heat check method (HCM method), on day 0 an intramuscular (IM) injection of 2 ml Lutalyse® was given followed 10 days after with another 2 ml of Lutalyse®, then check for signs of estrus on the 13^{th} – 14^{th} day prior to AI. HCM + GnRH method, same as described above except that 1 ml of Cystorelin® was given 18-24 hr before AI. Ov-Synch method, on day 0, an IM injection of 2 ml Lutalyse® was given, followed by 1 ml Cystorelin® on day 7^{th}. On day 14^{th} another 2 ml of Lutalyse® was injected IM, then followed up by another 1 ml Cystorelin® on day 17^{th} and AI (2x, AM – PM) and, CIDR method, on day 4 post estrus, CIDR was inserted deeply in the vagina of does using an applicator, the CIDR unfolds into a "T" like formation that aids in retention (for 15 days), before its removal followed by IM injection of 2 ml Lutalyse® and 1 ml Cystorelin®. AI was conducted on the 17^{th} day post estrus.

Estrus signs

Recognizable signs of estrus post treatment includes the following, a) restlessness and head butting b) tail flagging c) vaginal swelling d) presence of mucus discharge e) mounting and, f) pinkish to reddish color of the cervix upon examination.

Semen preparation for AI

Semen straws were thawed by dipping into a water container at 37°C - 39°C for approximately 20 sec before loading into AI tube. The upper portion of the

straw on the tip of the tube was cut before putting the AI plastic sheath and secured for AI.

AI method

The doe was kept tightly by two legs of the helper, and the hind legs lifted so that the rear part is kept higher than the fore part of the body. A lubricated vaginal speculum and/or a locally fabricated device with small bulb attached inside or a pen light/ flash-light was used to open the vaginal cavity and expose the cervix and os. The AI tube is then inserted deeply enough into the os and the semen deposited slowly.

Pregnancy diagnosis through ultrasonography

The ultrasound technique (Noveko 5.0 MHz) adopted was the B-mode or real-time transabdominal ultrasonography. Prior to examination, the right side of the abdomen just in front of the mammary gland was shaved to remove the hair that may interfere with the data received by the ultrasound probe. Then, a Trans-Gel (ultrasound transmission gel – a water based contact medium for ultrasound transmission) was applied to ensure a clear projection of information to and from the probe. Examination was carried out between the 60^{th} – 90^{th} day post insemination.

Results and Discussion

Oestrus response to treatments

Among goats treated for ES using different approaches, the various manifestations of "heat" observed showed no significant difference (Table 1). The presence of mucus discharges were not always associated with the pinkish appearance/openness of the cervix but were always coincidental with vaginal swelling. Mounting, tail flagging, restlessness with head butting were observed in most doe(s). However, these common signs of "in-heat" doe(s) were not a determinant of doe(s) exact stage of estrus. Proper determination of estrus stage for AI could be done by mucus examination in the anterior portion of the vaginal canal through the use of speculum and good light source. Most of doe(s) internal mucus were observed to be clear, thin and very liquid/watery in consistency in the morning (too early for AI) which turns opaque, good elasticity with viscous consistency and form in the afternoon (just the right time

for AI). Doe(s) found/observed with white, pale or yellow mucus discharges that were thick and lacks elasticity were deemed late for AI.

Overall, the estrus response of treated doe(s) regardless of the methods adopted were similar to reports when using Ov-Sync, intravaginal progestagen + eCG or prostaglandin alone (Ishwar and Pandey, 1990; Holtz et al., 2008; Ahmad et al., 2011).

Table 1. Manifestation of estrus signs following different ES methods*

Methods	No. of goats (%)					
	Treated	Responded	Mucus discharge	Pink/Open cervix	Vaginal swelling	Head butting/mounting tail flagging
HCM	10	8 (80.0)	8	7	8	8
HCM + GnRH	10	7 (70.0)	7	6	7	7
Ov Synch	10	10(100.0)	10	7	10	9
CIDR®	10	9 (90.0)	9	8	9	8

*48 hr observation period.

Pregnancies following AI

A satisfactory kidding rate has been reported when using PGF_2 alpha alone/or with progesterone (Ishwar and Pandey, 1990) or supplementation at the time of insemination with GnRH (Kleeman et al., 1994; Lashari and Tashawar, 2007). Administration of GnRH at the time of breeding was said to enhance fertility (McLeod et al., 1982), induced ovulation (Southee et al., 1982) and enhance subsequent luteal phase (Acosta et al.,2003) for pregnancy maintenance. In this study, the pregnancy rate obtained with the use of PGF_2 alpha alone/ or in combination with GnRH had a kidding rate of 50.0% (Table 2). Others have reported an enhanced fecundity when using a combination of GnRH-PGF_2 alpha and the addition of progestagen sponges at the time of GnRH administration tend to improve reproductive parameters (Titi et al., 2010). The use of CIDR® method in Boer and Boer-crosbred doe(s) has resulted to 40.0 % pregnancy rate. This observation was comparable to the output of NCSU group of researchers using CIDR-AI for Boer goat reproduction obtaining a 45.0% pregnancy rate (Farin et al., 2013). Moreover, when using NC-Synch method, their pregnancy rate ranged from 9.0 – 52.0 %. In contrast, using Ov-Synch method, a precursor of NC-Synch method, we obtained 0.0% pregnancy rate. Among the factors deemed to have influenced

the totality of the results gathered include the quality of semen used and the timing of AI.

During non-breeding season, hormone treatments based on progestagens and eCG were claimed to provide good pregnancy rates for AI undertaken at a fixed time point (Boscos et al., 2002; Lopez-Sebastian et al., 2007). Other researchers recommended the use of progestagen vaginal sponge and/or CIDR combined with eCG-cloprostenol injections to achieve synchronization in goats of both temperate and tropical zones (Freitas et al., 2004). In this study, the application of ES methods during non-breeding season achieved a satisfactory estrus induction and fecundity rate. The application of similar ES approaches during the mid-breeding season (Boscos et al., 2002) and deep anoestrus period (Pellicer-Rubio et al., 2008) appears equally effective at the beginning of the breeding season with no case of abnormal ovarian response.

Nonetheless, ES currently used for goats in tropical regions were developed for goats bred in temperate regions. Thus, several alternative considerations for improving the efficiency of hormonal treatment need to be evaluated. Of utmost importance is the availability of nutrients as the ultimate regulator of reproduction in the tropics since the control of nutritional condition is essential before the use of hormonal treatment for ES/AI in goats. An alternative approach is the development of new breeding strategies for use in countries of the tropics, based on buck teasing/buck effect, to control estrus and ovulation in combination with AI, to reduce dependency on the use of hormones. With the development of AI using frozen-semen, a classical selection program could be arranged including planned mating, progeny testing and the diffusion of proven sires by insemination in a given herds.

Lastly, the cost of hormonal treatment per animal need to be evaluated for practical reasons especially if the technique(s) is to be applied and recommended for use by the farmers in the tropics. Based on our experience, the use of PGF_2 alpha alone at a cost of 100.00 pesos/animal can be considered reasonable/acceptable for farmers to adopt. Technology adoption by the farmers will not only help increase the overall productivity of their livestock but its resulting products (eg., meat and milk) and income as well.

Table 2. Conception rate after AI*.

Methods	No. of goats (%)		No. of kids	Cost per animal** (Peso)
	Inseminated	Pregnant		
HCM	10	3 (30.0)	6	100.00
HCM + GnRH	10	2 (20.0)	4	335.00
Ov Synch	10	0	0	670.00
CIDR	10	4 (40.0)	7	1,100.00

*Pregnancy detected through ultrasonography: **Based on prevailing market price (2015).

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