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SERUM PROGESTERONE PROFILES OF SAHEL DOES TREATED WITH PROGESTAGENS WITH OR WITHOUT EXPOSURE TO BUCK EFFECT

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ABSTRACT

An enzyme-linked immunosorbent assay (ELISA) was carried out to measure serum progesterone (P_4) concentrations in homogenous groups of Sahel (SH) does treated with different progestagens with or without exposure to bucks. SH does were treated with: I - aproned buck exposure and CIDR (ABECD), II - no buck exposure and CIDR only (NBECD), III - aproned buck exposure and FGA-30 mg (ABEF3), IV - no buck exposure and FGA-30 mg only (NBEF3), V - aproned buck exposure and FGA-45 mg (ABEF4), VI - no buck exposure and FGA-45 mg only (NBEF4). Serum P_4 was determined on days 0 (progestin insertion), 3, 7, 10, 14 (progestin withdrawal) and oestrus. Overall, mean P_4 concentration on day 0 was 1.63, 7.92, 0.75, 1.89, 0.24 and 8.79 for the ABECD, NBECD, ABEF3, NBEF3, ABEF4 and NBEF4 groups, respectively. An increase in P_4 levels was recorded in the ABECD does by day 4, followed by peak P_4 level (4.03 ng/ml) on day 7. Serum P_4 levels decreased generally from pre-insertion levels to basal levels during oestrus. At oestrus, mean serum concentration was 0.40 ng/ml with no significant difference between groups. Male presence did not significantly alter P_4 levels across the days of treatment. It is concluded that progestagen rather than male presence influenced serum P_4 levels in Sahel does.

Keywords: male effect; progesterone profile; progestagens; goat

INTRODUCTION

Oestrus synchronisation (OS) is an assisted reproductive technique that focuses on the manipulation of the oestrus cycle in livestock and can be achieved using prostaglandins, progestagens, gonadotrophins and the male effect (Abecia *et al.*, 2012). The success of an OS may be influenced by the cyclicity and stage of the oestrus cycle, nutrition, gonadotrophin use, season, breed, presence of a male, type and duration of treatment (Avdi *et al.*, 2004; Lehloenya *et al.*, 2008). These factors including genetic and environmental factors affect the reproductive efficiency of the female goat. Reproductive efficiency is critical in goat meat and milk production. Prostaglandins act by causing luteolysis; while exogenous progesterone administration lengthens the luteal phase of the oestrus cycle, thereby exerting a negative feed-back on the pulse frequency of the gonadotrophin-releasing-hormone (Medan *et al.*, 2005; Fatet

et al., 2011). The use of long-term progesterone treatment is effective in synchronizing oestrus in ruminants. However, daily administration of progesterone is impracticable under field conditions because it is very labour intensive. Newer methods of progesterone delivery include oral, subcutaneous ear implants, progesterone pessaries and the use of controlled internal drug release (CIDR) device (Romano, 1994; Kawu *et al.,* 2003). Assessment of progesterone levels during oestrus synchronisation using different progestagens allows for the evaluation of bioavailability and concentrations of different agents, which, in combination with the stage of the oestrus cycle, are important parameters to determine oestrus response, behaviour and fertility in ruminants (Oladimeji *et al*., 2001; Zarkawi, 2000; Vilarino *et al*., 2010). Studies have also described the progesterone profile of females after progestagen and/or prostaglandin administration in cows (Rekwot *et al.,* 2004), sheep (Vilarino *et al.,* 2010), sows (Willis *et al.,* 2003), equines (Amer *et al*., 2008), buffalos (Perera, 2011), dogs (Concannon, 2011) and goats (Riesenberg *et al*., 2001). Some studies have reported the plasma and serum P_4 levels during the oestrus cycle, gestation and postpartum period of different breeds of goats (Kawu *et al.,* 2004; Gaafar *et al.,* 2005). However, there is a paucity of information on serum concentrations of progesterone levels in the Sahel breed of goat; and the effects of different progestagens on progesterone profile of does exposed to the male effect during oestrus synchronisation. The aim of this study was to determine the progesterone profile of Sahel does during oestrus synchronisation using different progestagens, with or without exposure to buck stimulus.

MATERIALS AND METHODS

Location of Study

The study was carried out at the goat farm of the Small Ruminant Research Programme, National Animal Production Research Institute (NAPRI), Shika, Ahmadu Bello University, Zaria. NAPRI is located in the Northern Guinea Savannah of Nigeria between latitude 11 $^{\circ}$N and 12 $^{\circ}$N and between longitude 7 $^{\circ}$E and 8 $^{\circ}$E at an elevation of 650 m above sea level. The hormonal assays were carried out at the research laboratory of the Animal Reproduction Research Programme of NAPRI. NAPRI is located in the Northern Guinea Savannah zone of Nigeria between latitude 11^0 N and 12^0 N and between longitude 7^0 E and 8^0 E at an elevation of 650 m above sea level with an average annual maximum and minimum temperature of 31.0 ± 3.2 ^{o}C and 18.0 ± 3.7 ^{o}C respectively. Shika has anaverage annual rainfall of 1100 mm usually lasting from May to October with a mean relative humidity of 72 % while the dry season lasts from November toApril with mean daily temperatures ranging from 15 – 36 ^{o}C and mean relative humidity of between 20 –37 % (Rekwot *et al.,* 2004).

Experimental Animals and Herd Management

Sahel (SH) does with body condition score (BCS) range of 2.5-3.5 (Mellado *et al.,* 2004) and aged 2-3 years were used in this study. Does were identified by means of plastic ear tags, screened for blood and helminthes parasites, and appropriate treatments and vaccinations were carried out. Does were fed *Digitaria smutsii* hay and concentrate supplement (0.5 kg/head/day) and grazed native pastures during the day time. They were given access to water *ad libitum*. The does were housed in pens with large paddocks during the daytime for grazing. Does were dipped once weekly during the dry season and twice weekly during the rainy season in Coumaphos (Asuntol®, Bayer) an organophosphate acaricide at a dilution of 1 litre of 16 % coumaphos to

800 litres of water for the control of ectoparasites. Does that kidded more than five months earlier had their kids withdrawn at least one month before commencement of the study.

Experimental Design

SH does (n = 18) were randomly allocated into six groups (n = 3/group): (1) aproned buck exposure and controlled internal drug release (CIDR) treatment (ABECD); (2) no-buck exposure and CIDR treatment only (NBECD); (3) aproned buck exposure and fluorogestone acetate (FGA)-30 mg treatment (ABEF3); (4) no buck exposure and FGA-30 mg treatment (NBEF3); (5) aproned buck exposure and FGA-30 mg treatment (ABEF3) and (6) no buck exposure and FGA-30 mg treatment only (NBEF3) (Table 1). Intravaginal progestagens (CIDR, FGA-30 mg or FGA-45 mg) were inserted for 14 days with or without buck exposure on day 7 to the end of progestagen treatment. The CIDR is a T-shaped intravaginal implant, containing 300 mg natural progesterone (Eazi-Breed CIDR, InterAg, Hamilton, New Zealand) and impregnated in a silicon elastomer core. The FGA-30 mg and FGA-45 mg are intravaginal sponges impregnated with 30 and 45 mg FGA (Chronogest, Intervet S.A., Angers, France), respectively. A minimum distance of about 500 m was used to separate the buck exposed (ABE, ABECD, ABEF3, ABEF4) from the no-buck exposed (NBECD, NBEF3. NBEF4, NBECN) does so as to minimize influence of exteroceptive stimuli, associated with each other. Intravaginal progestagens were withdrawn on day 14 post-insertion (Figure 1). Following removal, does were placed for five days with intact bucks to detect oestrus.

Table 1: Group summary of Sahel does treated with exogenous hormones with or without exposure to bucks

Group	Treatments	n
ABECD	Aproned buck exposure and CIDR	3
NBECD	No buck exposure and CIDR	3
ABEF3	Aproned buck exposure and FGA-30 mg	3
NBEF3	No buck exposure and FGA-30 mg	3
ABEF4	Aproned buck exposure and FGA-45 mg	3
NBEF4	No buck exposure and FGA-45 mg	3

n = Number of does

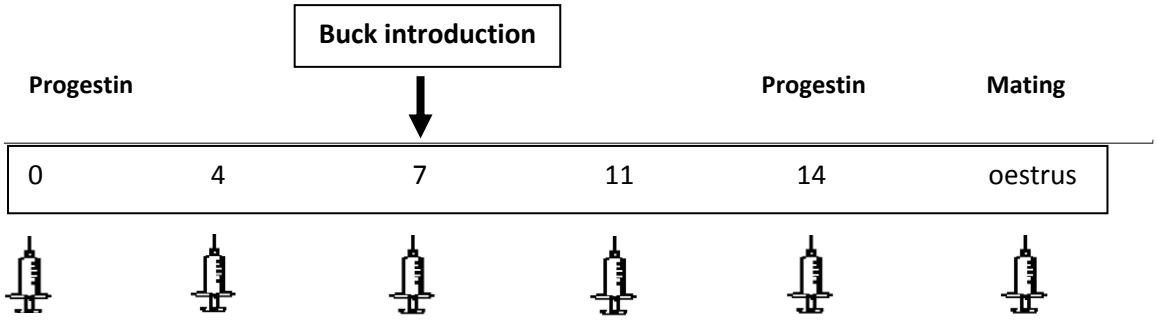


Figure 1: Blood sampling summary to evaluate serum progesterone profile of Sahel does treated with CIDR®, FGA-30® and FGA-45® with or without aproned buck exposure.

CIDR® = Controlled internal drug release devices
FGA-30® = 30 mg fluorogestone acetate sponges
FGA-45® = 45 mg fluorogestone acetate sponges

Sample Collection and Hormonal Assay

Five milliliters of blood were collected at 08:00 hours by jugular venipuncture into sterile plain bottles using a 10 mL hypodermic syringe, fitted with 21 gauge needle on days 0 (before insertion of progestagen), 4, 7 (day of buck exposure), 11, 14 (day of progestagens withdrawal) and oestrus. Blood samples were allowed to clot, quickly transported to the laboratory and stored at +4 ^{o}C for 24 hours. Thereafter, serum samples were separated by centrifugation at 2500 x g for 15 minutes, placed in appropriately labeled serum vials and stored at -20˚C until analyses. Concentration of progesterone was measured in serum using competitive immune-enzymatic colorimetric method for quantitative determination of progesterone. The method is based on the principle of solid phase enzyme-linked immunosorbent assay (ELISA). Accu-bind® (Monobind-Lake Forest, CA 92630 USA) ELISA kits were used according to the manufacturer's specifications.

Data Collection and Analyses

Serum levels in ng/mL were expressed as mean ± S.E.M. One-way analysis of variance and Tukey's post-hoc test was used to compare means among groups. Student's t-test was used to compare serum levels between successive days of sampling. Graphpad Prism® data package version 5.0 for Windows (2009) was employed for all statistical analyses. Values of $P < 0.05$ were considered significant.

Results and discussion

The serum progesterone levels of SH does are shown in Figure 2. Mean serum progesterone peaked, from basal levels on day 0 (day of progestagen insertion) to 3.15 ng/ml by day 4. Mean peak levels of serum progesterone were observed on day 11 of ABECD treatment, with a mean peak concentration of 4.03 ng/mL. The concentration fell to its lowest level on day 14 (final day of treatment) to a mean level of 2.99 ng/mL (Figure 2). The study corroborates other findings (Rowe and East, 1996; Freitas *et al.*, 1997) that progestagen treatments increased serum P_4 levels in goats. This was demonstrated by the changes observed in the steroid hormone secretion. This

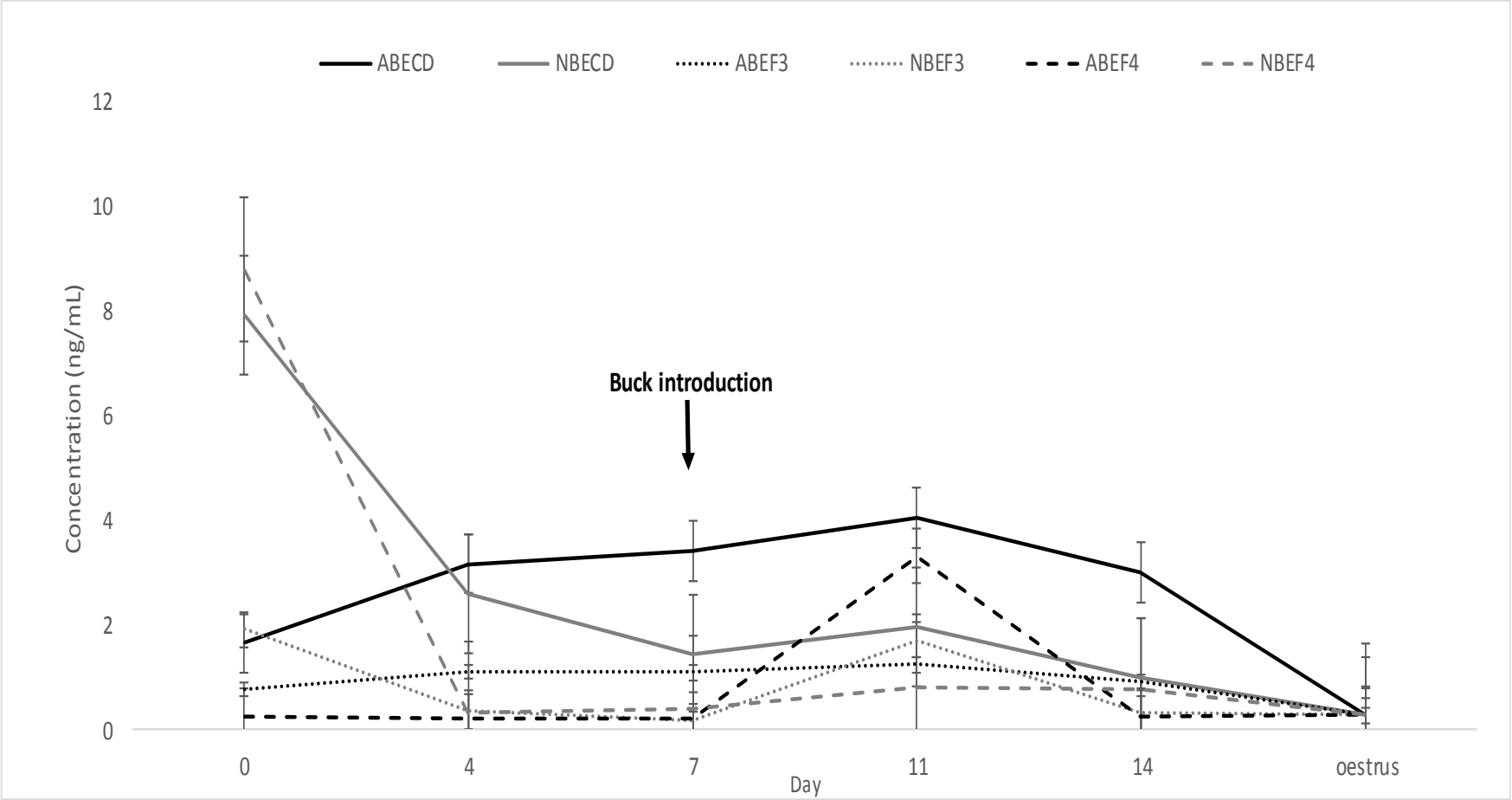


Figure 2: Mean progesterone concentrations of Sahel does during treatment with progestagens (CIDR, FGA-30 mg and FGA-45 mg) with or without exposure to bucks

ABECD = Buck exposure + CIDR NBECD = No buck exposure + CIDR ABEF3 = Buck exposure + FGA-30

NBEF3 = No buck exposure + FGA-30 ABEF4 = Buck exposure + FGA-45 NBEF4 = No exposure + FGA-

pattern of increase to maximum and then decline was similar to that recorded by others (Hamra *et al*., 1989; Wheaton *et al*., 1993; Ali *et al.*, 2009) observed peak plasma progesterone levels by day 4 - 6 of treatment using CIDR devices, with declining levels to < 0.4 ng/mL on day 12 of treatment. Kauser *et al.* (2005) reported similar findings of peak plasma progesterone concentrations during the mid-luteal phase of the oestrus cycle in dwarf goats.

The increase in serum progesterone levels recorded in all groups was characteristic for intravaginal progestagens insertion in goats (Wheaton *et al*., 1993; Ali *et al.*, 2009). Overall, serum progesterone concentration decreased from 3.54 ng/mL to 1.01 ng/mL by day 7 and remained stable till the end of progestagen insertion (day 14). Generally, in the present study, serum P_4 levels declined in all groups by day 14 of progestin insertion. Low detectable P_4 levels during oestrus may be due to the fact that ovarian follicles also produce progesterone, apart from estrogens (Singh *et al.*, 1999). The peri-ovulatory period in goats is characterized with low values of progesterone (below 1ng/mL) and the current results obtained corresponded with values of other researchers (Mori and Kano, 1984; Błaszczyk *et al.*, 2004). Lowest P_4 levels were observed at day of oestrus, indicating minimal luteal activity. Dose of intravaginal progestin did not influence serum progesterone profiles and concentrations. Similarly, buck exposure had no influence on P_4 levels in Sahel does. It is worthy of note that irrespective of the type of progestagen used, a 14-day treatment protocol was adequate to initiate the mechanisms responsible for the manifestation of oestrus.

CONCLUSIONS

Exposure of SH does to males at the end of different progestagen treatments did not significantly affect serum P_4 levels. Both doses of fluorogestone acetate showed a similar declining pattern in P_4 concentrations from time of insertion. The P_4 levels in CIDR-treated does rose to peak values at day 4 before declining to basal levels during oestrus. Further studies are required evaluate the changes in steroid hormone concentrations after a longer period of progestagen treatment.

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CONFLICT OF INTERESTS

The authors have none to declare.

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