Research Note

EVALUATING A PROTOCOL FOR FIXED TIME ARTIFICIAL INSEMINATION USING TWO PROGESTERONE IMPLANTS IN CROSSBRED EWES RAISED UNDER HEAT STRESS[1,2]

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Assisted reproduction technologies, including fixed-time trans-cervical artificial insemination (TAI), are main components in sheep improvement programs. However, TAI requires synchronizing heat in ewes using exogenous hormones at specific time intervals since its purpose is to maintain a uniform reproductive cycle within the ewe during the year. In tropical climates with temperature-humidity index (THI) above the livestock comfort zone ewes live in constant thermal stress that may alter their physiological response to exogenous hormones. The objective of this experiment was to evaluate a protocol for fixed-time artificial insemination using two progesterone implants in crossbred ewes raised under the thermal stress of Puerto Rico. The experiment was conducted in The Small Ruminant Project at the University of Puerto Rico, Mayagüez Campus. Twelve crossbred ewes (34.16 kg) raised under thermal stress conditions (THI>72) were used to evaluate a protocol using two progesterone implants (Figure 1).

Ewes were treated with prostaglandin, $PGF_2\alpha$ (2 ml, IM), 48 h before being implanted with either a controlled internal drug release (CIDR, n=6) containing 300 mg of progesterone or with an intra vaginal sponge (n=6) containing 60 mg of progesterone for a period of 12 days. After withdrawal of the devices (CIDR/sponge), the animals were injected with 400 IU of PMSG (pregnant mare serum gonadotropin) and 200 IU of HCG (human chorionic gonadotropin). In all ewes, TAI was executed three times using fresh semen: 47, 59 and 71 hours after the gonadotropin injections. Jugular blood samples were collected from the ewes before placing the implants, 8 d after implanted with the CIDR or sponge, and 68 d after TAI. Blood plasma was harvested and stored at 20° C until analyzed for progesterone concentrations. To determine the physiological status of the ewes, respiratory rate (RR), heart rate (HR) and rectal temperature (RT) measurements were taken four times (0 d, +8 d, +14 d and +84 d) within the protocol. These measurements were compared to those of animals that were in their comfort zone to prove thermal stress conditions in the animals.

Data on the effect of the CIDR or sponge on ewe progesterone levels 8 d after placing the implant and after its removal were analyzed using a completely randomized design (SAS, 2009). Tukey-T was used for mean separation. Fertility (pregnant/inseminated

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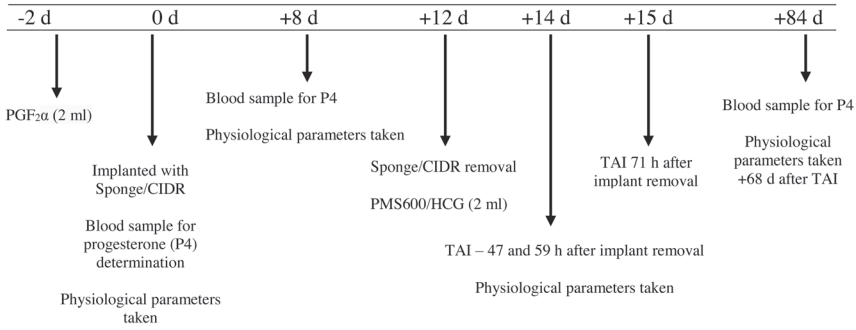

FIGURE 1. Fixed-time trans-cervical artificial insemination (TAI) protocol by day using progesterone implants in ewes.

ewes), prolificacy (multiple fetuses/pregnant ewe), and fecundity (fetuses/inseminated ewe) were determined. Pregnancy was diagnosed based on ewe plasma progesterone levels 68 d after TAI, where: no pregnancy = < 2.5 ng/ml, one fetus = 2.6 – 8.0 ng/ml, and multiple fetuses = > 8 ng/ml.

Ewes are homoeothermic animals and need to maintain a constant body temperature. They are sensitive to factors that influence their thermal exchange with the en-

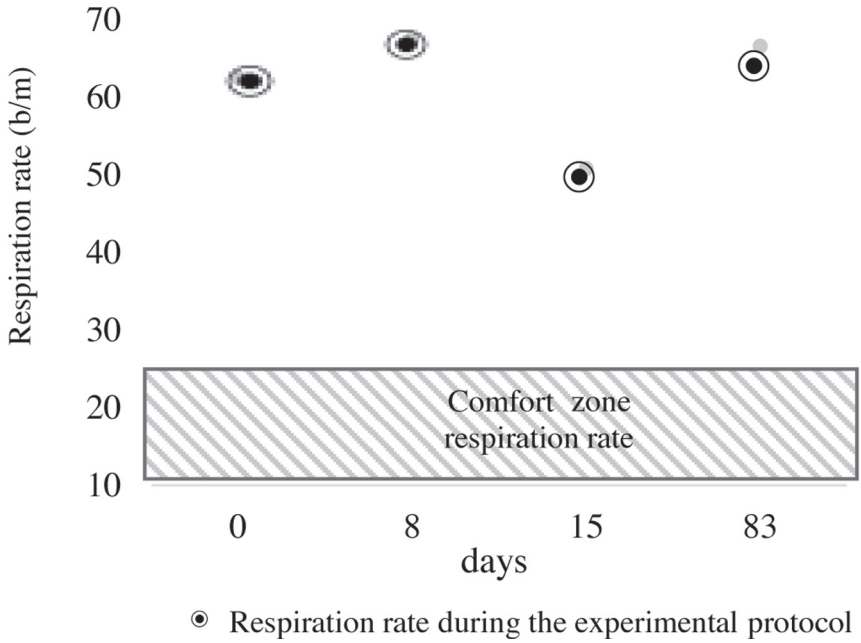

FIGURE 2. Respiration rate of ewes during the protocol compared to values within the comfort zone.

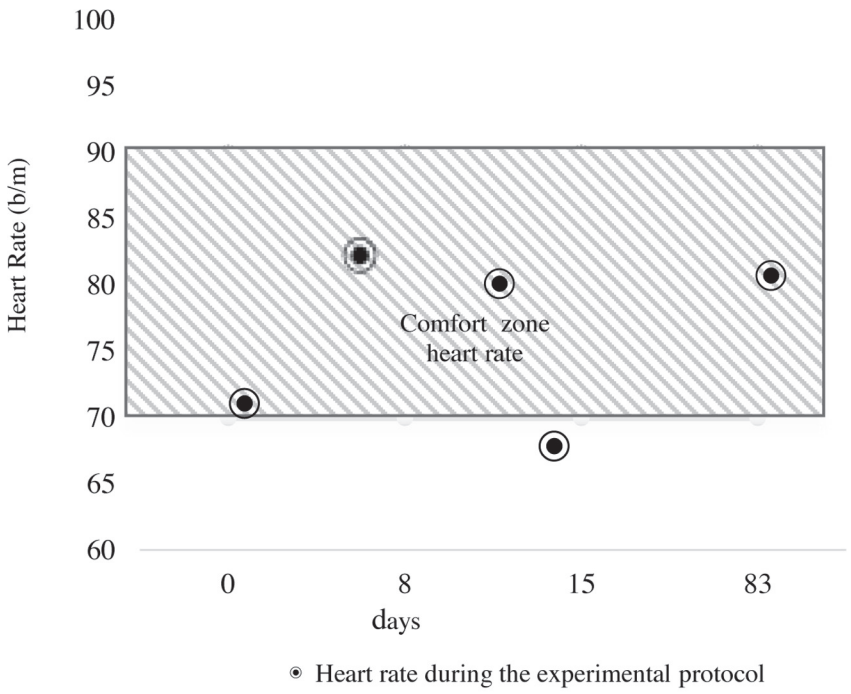

FIGURE 3. Heart rate of ewes during the protocol compared to values within the comfort zone.

vironment, including air temperature, radiant temperature, air velocity, and relative humidity. In tropical environments the animals are normally raised in conditions above the comfort or thermoneutral zone and THI is usually higher than 72. At temperatures above the comfort zone ewes increase their respiration rate in an attempt to increase heat dispersion, an observation which coincides with findings of this experiment (Marai et al., 2007). Results show that RR of ewes exposed to thermal stress was above the values reported for animals in their comfort zone (Figure 2). However, HR and RT were under or within the normal values (Figures 3 and 4).

As shown in Table 1, in this experiment, the level of progesterone in ewes under thermal stress was similar for both types of implants (0.314 and 0.517 ng/ml for CIDR and sponge, respectively) after two days of $PGF_2\alpha$ injection. However, eight days after estrous synchronization, progesterone levels were higher ($P < 0.05$) in ewes with CIDR implants than those treated with sponges (3.92 vs. 2.40 ng/ml, respectively), values which may be associated with the hormone concentration and release of the implant (300 vs. 60 mg for CIDR and sponge, respectively).

The overall pregnancy rate was 41.5% (5/12) as indicated in ewes with blood progesterone levels lower than 2.5 ng/ml (Table 2). Ewes implanted with a sponge averaged higher fertility (50%) than those with CIDR (33%), but lower prolificacy (two fetuses per pregnant ewe vs. one) and fecundity (50% vs. 66% for sponge and CIDR, respectively) (Table 3). Fertility, prolificacy and fecundity values found in our research are similar to research related to TAI in sheep (Fleisch et al., 2012; Karaca et al., 2009).

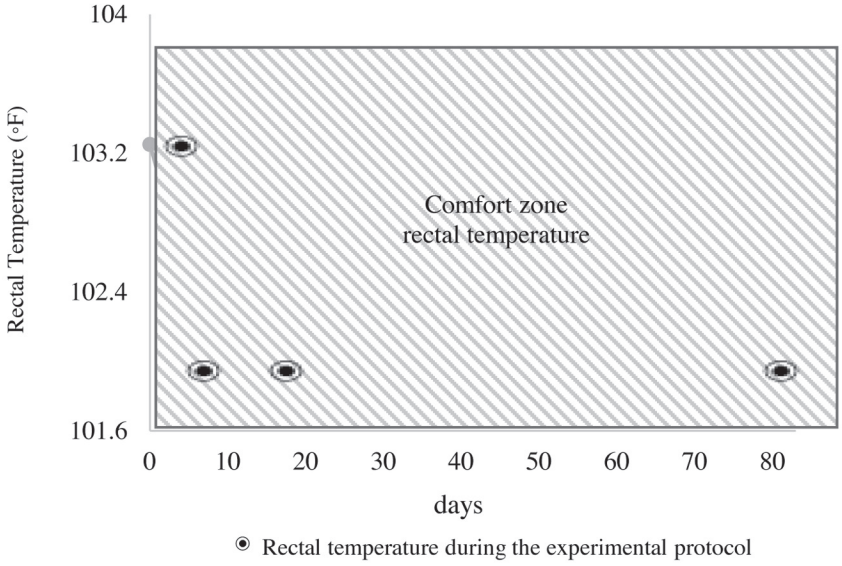

FIGURE 4. Rectal temperature of ewes during the protocol compared to values within the comfort zone.

TABLE 1.—*Progesterone levels in ewes implanted with a controlled internal drug release (CIDR) or sponge under thermal stress.*

		ng/ml		
Day	N	CIDR	Sponge	P
0	6	0.314	0.517	3.014
8	6	3.92	2.40	0.048

TABLE 2.—*Progesterone level (ng/ml) in ewes 68 days after TAI. Values in bold indicate pregnancy*.

N	CIDR	Sponge
1	0.943	**5.34**
2	1.25	0.314
3	**13.20**	0.628
4	2.43	**4.71**
5	1.57	**5.03**
6	**13.83**	0.314

TABLE 3.—*Fertility, prolificacy and fecundity in ewes implanted with a controlled internal drug release (CIDR) or sponge.*

	Implant	
	CIDR	Sponge %
Fertility	33	50
Prolificacy	200	100
Fecundity	66	50

These results indicate that TAI procedures in ewes implanted with CIDR or sponge can be used in ewes raised under thermal stress without adversely affecting fertility. Due to a lack of seasonal anestrous, these procedures have the potential for year-round use in situations similar to that of the present study.

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