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# Alternatives to improve a prostaglandin-based protocol for timed artificial insemination in sheep

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**Abstract**

The objective was to improve the reproductive performance of a prostaglandin (PG) $F_{2\alpha}$-based protocol for timed artificial insemination (TAI) in sheep (Synchrovine®: two doses of 160 μg of delprostenate 7 d apart, with TAI 42 h after second dose). Three experiments were performed: Experiment 1) two doses of a $PGF_{2\alpha}$ analogue (delprostenate 80 or 160 μg) given 7 d apart; Experiment 2) two $PGF_{2\alpha}$ treatment intervals (7 or 8 d apart) and two times of TAI (42 or 48 h); and Experiment 3) insemination 12 h after estrus detection or TAI with concurrent GnRH. Experiments involved 1131 ewes that received cervical insemination with fresh semen during the breeding season (32/34 °S–58 °W). Estrous behaviour, conception rate, prolificacy, and fecundity (ultrasonography 30–40 d), were assessed. In Experiment 1, ewes showing estrus between 25 and 48 h or at 72 h after the second $PGF_{2\alpha}$ did not differ between 80 and 160 μg of delprostenate (73 vs 86%, $P = 0.07$; and 92 vs 95%, P = NS, respectively). Conception rate and fecundity were lower ($P < 0.05$) using 80 vs 160 μg (0.24 vs 0.42, and 0.27 vs 0.47, respectively). In Experiment 2, giving $PGF_{2\alpha}$ 7 d apart resulted in higher ($P < 0.05$) rates of conception (0.45 and 0.51) and fecundity (0.49 and 0.53) than treatments 8 d apart (conception: 0.33 and 0.29; fecundity: 0.33 and 0.34) for TAI at 42 and 48 h, respectively. In Experiment 3, rates of conception, prolificacy and fecundity were similar (NS) between Synchrovine® with TAI at 42 h (0.50, 1.13, and 0.56) and AI 12 h after estrus detection (0.47, 1.18, and 0.55), and Synchrovine® plus GnRH at TAI (0.38, 1.28, and 0.49). However, all TAI treatments had lower ($P < 0.05$) prolificacy and fecundity compared to AI following detection of spontaneous estrus (1.39 and 0.83, respectively). In conclusion, the Synchrovine® protocol was: a) more successful using 160 vs 80 μg delprostenate; b) more successful with a 7 d than 8 d $PGF_{2\alpha}$ interval; c) similarly effective for TAI versus AI 12 h after estrus detection; and d) not improved by giving GnRH at TAI.


*Keywords:* Ewes; Estrus synchronization; AI; Conception

## 1. Introduction

Timed artificial insemination (TAI) is an important tool when estrus detection is not feasible. It provides synchronized inseminations and more efficient use of superior males [1]. Currently, widespread application of these biotechnologies under commercial field conditions requires easy implementation procedures, acceptable pregnancy rates, and low environmental impact [2]. Conventional TAI protocols involve intravaginal devices impregnated with progestins, in conjunction with eCG, yielding acceptable pregnancy rates both within and outside the physiological breeding season [3,4]. Nevertheless, repeated use of eCG has been as-

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sociated with a humoral immune response in ewes and goats [5,6] and development of ovarian follicular cysts [7], followed by low pregnancy rates. In addition, use of a progestin and eCG has been limited in some countries due to public health concerns [8] and animal welfare issues [9]. However, prostaglandin-based protocols may be a viable alternative to the use of progestagens.

Conventional $PGF_{2\alpha}$ treatments consist of two doses given 9 to 12 d apart [4]. Using this protocol, a high proportion of ewes are detected in estrus after the second dose, but over a 4–5 d interval [10,11], making estrus detection necessary and rendering this protocol inappropriate for TAI. Conversely, Rubianes et al [12] and Contreras-Solis et al [13] demonstrated that the refractoriness of a recently formed ovine CL to $PGF_{2\alpha}$ might be restricted to the first 2 d after ovulation, with a consistent interval from treatment to ovulation (60.8 ± 1.8 or 61.1 ± 1.1 h, respectively). Based on these observations, a protocol to synchronize estrus and ovulations (Synchrovine®, MIEM - Cámara Nacional de Registros, Montevideo Uruguay), with two large doses of $PGF_{2\alpha}$ given 7 d apart was proposed [1]. Synchrovine® promotes highly synchronous estrus during the first 72 h, with 80% of the ewes in estrus within 25 to 48 h after the second $PGF_{2\alpha}$ dose, making it practical for use in TAI programs. However, this protocol yielded lower conception rates after cervical or intrauterine TAI compared to the conventional P4-eCG protocol [14] or spontaneous estrus [15]. Thus, other alternatives to improve this protocol need to be studied.

A substantive reduction of the cloprostenol dose was effective in inducing luteolysis, estrus onset, ovulation, and subsequent development of normal CLs [16,17]. The reduced dose might decrease side effects, including changes in cervical mucus and uterine contractions [18,19]. A longer interval between $PGF_{2\alpha}$ treatments increased the sensitivity of young CLs to this hormone [20] and increased progesterone concentrations during development of the designated preovulatory follicle. When feasible, AI associated with estrus detection may be used to improve Synchrovine® results. However, a protocol including GnRH treatment 36 h after $PGF_{2\alpha}$ resulted in an LH surge, ovulation within 48 h, and a fully functional CL [21]. Therefore, administration of GnRH at AI would be a practical option if it were to improve ovulation and fertility, as demonstrated in dairy cows [22,23].

The aims of the present study were to determine whether either a reduction of $PGF_{2\alpha}$ luteolytic dose, an increase in the interval between $PGF_{2\alpha}$ treatments, AI of ewes detected in estrus, or giving GnRH at TAI, improved reproductive outcomes for a Synchrovine® synchronization protocol.

## 2. Materials and methods

### 2.1. Animals

All procedures were approved by the University's Animal Experimentation Committee (CHEA-UdelaR). The studies were done during the physiologic breeding season (March–April), involving 1131 clinically healthy, nulliparous and multiparous ewes (1.5–5.5 y old), with good body condition (score 3.4 ± 0.4, mean ± SD [24]), grazing natural pastures at three locations (Experiment 1: "El Coraje" Farm, Lavalleja Uruguay, 34 °S/56' W; Experiment 2: "Piedra Mora" Farm, Paysandú Uruguay, 32 °S/ 57' W; and Experiment 3: "Mario A. Cassinoni" Experimental Station, Paysandú Uruguay, 32 °S/ 58' W). On these farms, 13 clinically healthy rams aged 1.5–5.5 y old, grazing natural improved pastures (with lotus and rye grass) were used as semen donors.

### 2.2. Experiments

Three experiments were done. Ewes and rams were either randomly assigned to treatments (Experiment 1 and 2) or allocated to treatments, on the basis of breed, parity, and body condition score (Experiment 3).

#### 2.2.1. Experiment 1

Corriedale multiparous ewes (n = 127) were subjected to cervical TAI (April 12 and 13, Year 1) with fresh semen from two Corriedale rams. Two $PGF_{2\alpha}$ doses for Synchrovine® were compared:

- *Synchrovine®*: Two doses of delprostenate (160 μg im; Glandinex®, Universal Lab, Montevideo, Uruguay) given 7 d apart, with TAI 42 h after the second dose (Control).
- *Synchrovine®-LD*: As above, but using 80 μg delprostenate.

#### 2.2.2. Experiment 2

Australian Merino multiparous ewes (n = 583) were cervically TAI (April 11 and 12, Year 2), with fresh extended semen from five rams (three Australian Merino and two Suffolk). In a factorial design, two $PGF_{2\alpha}$ treatment intervals (7 or 8 d) and two AI-times (42 or 48 ± 1 h after the second dose of $PGF_{2\alpha}$) for Synchrovine® were compared.

- *Synchrovine®*: as described in Experiment 1.
- *Synchrovine®-48*: as above, with TAI at 48 h after second dose.