



# Effect of stimulation with a gonadotropin mixture on reproductive outcome in nulliparous ewes bred during seasonal anestrus and early breeding season

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## ABSTRACT

To evaluate the effect of gonadotropic stimulation on the reproductive response of nulliparous acyclic female sheep during mid-anestrus and early breeding season, fall-born ewe lambs of mixed breeding ( $N = 191$ ; ~7 months old; study 1) and yearlings (Dorset X Texel (DT),  $N = 120$ , study 2) were assigned randomly during May to July to be either pre-treated with progesterone using CIDR-g (0.3 g of progesterone) devices for 5 days or to also receive gonadotropins (240 IU eCG and 120 IU hCG, 3 mL i.m. injection of P.G. 600®) at CIDR-g removal. Study 3 was conducted with nulliparous acyclic DT yearling ewes ( $N = 104$ ) in late August and was similar to study 2 except that a group of control ewes that were not treated was included. Gonadotropic stimulation increased estrous response ( $P = 0.0002$ ), pregnancy rate to the first service period ( $P = 0.0007$ ), proportion of ewes lambing ( $P = 0.03$ ) and the lambing rate ( $P = 0.01$ ) in fall-born ewe lambs (study 1), but did not modify reproductive outcome in yearling females (study 2) during mid-anestrus. During the transition into the breeding season (study 3), progesterone pre-treatment increased the pregnancy rate and the proportion of ewes lambing to first service after ram introduction ( $P = 0.003$  and  $P = 0.02$ , respectively). However, gonadotropic stimulation of progesterone pre-treated yearlings did not modify reproductive performance variables. In conclusion, gonadotropic stimulation improved reproductive outcome in fall-born ewe lambs bred during the mid-anestrus period, but did not affect reproductive outcome in yearling females.

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## 1. Introduction

Ewes that lamb at one year of age produce 20% more lambs than their flock mates that are bred first at 16–18 months of age (Spencer et al., 1942; Hohenboken et al.,

1977). The length of the breeding season is shorter (Hafez, 1952; Quirke et al., 1985) and fertility of spring-born ewe lambs bred during the breeding season is lower than adult flock mates (Quirke, 1978; Gaskins et al., 2005), which reduces the proportion of ewes that lamb at one year of age.

Lambs born in the fall reach the age and weight associated with the commencement of puberty in their spring-born counterparts during the anestrus period but do not show regular estrous cycles until the subsequent fall when they are almost one year of age (Vesely and Swierstra, 1987; Forcada et al., 1991). Further, fall-born ewe lambs derived from parents selected to breed year round did not conceive during seasonal anestrus the next spring (al-Shorepy and Notter, 1996).

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The ram-induced secretion of gonadotropins is necessary for increased production of estrogen, the expression of estrus and ovulation in progesterone pre-treated anestrus ewes (Knights et al., 2001a,b). Attempts to induce fertile mating during anestrus in fall-born ewe lambs and yearlings with ram introduction (López-Sebastian et al., 1985) or with progestogen pre-treatment alone (Burfening and Van Horn, 1970) have not been successful. Abrupt introduction of rams to fall-born ewe lambs during anestrus activated the hypothalamic-pituitary gonadal axis as reflected by increases in LH secretion, follicle growth and ovulation but fewer than 50% of progesterone pre-treated fall-born ewe lambs exhibited estrus (Knights et al., 2002).

Treatment with equine chorionic gonadotropin (eCG) after progestogen priming induced fertile estrus in fall-born ewe lambs (Ainsworth and Shrestha, 1987; Ainsworth et al., 1991; Stellflug et al., 1993) and yearlings (Robinson and Smith, 1967; Burfening and Van Horn, 1970) bred during seasonal anestrus. Additionally, a small dose of estrogen increased the proportion of progesterone-primed fall-born ewe lambs expressing estrus during anestrus (Knights et al., 2002). These findings indicate that supplementation of the ram-induced release of gonadotropins might be necessary to stimulate sufficient amounts of estrogen to induce fertile estrus in progesterone pre-treated fall-born ewe lambs and spring-born yearlings.

Stimulation with eCG after progestogen priming also induced fertile estrus and advanced ages at first conception and lambing in spring-born lambs (Stellflug et al., 2001; Sawalha et al., 2011). Those data led to the suggestion that gonadotropic support might improve reproductive performance of nulliparous ewes bred during the breeding season. However, those studies did not include nulliparous ewes treated with progestogen only, which precluded a determination of a specific requirement for gonadotropic stimulation.

Gonadotropic and estrogenic preparations are not currently approved for use in sheep in the United States. P.G. 600® a gonadotropic preparation containing 400 IU of eCG and 200 IU of hCG is approved for use in swine and treatment with a controlled intravaginal/internal drug releasing (CIDR) device containing 0.3 g progesterone for 5 days is approved for induction of estrus in sheep (Knights et al., 2001a,b; 2003). A 5-day pre-treatment with progesterone prior to introduction of rams was previously shown to be as effective as a 12-day treatment in inducing fertile estrus in anestrus ewes (Knights et al., 2001a) and has subsequently been demonstrated to be effective in other studies (Knights et al., 2001b; 2003; D'Souza et al., 2014). The aim of the present study was to determine the effect of gonadotropic stimulation on fertility of nulliparous ewes pre-treated with progesterone using CIDR devices during the mid- and late-anestrus period at the transition into the breeding season.

## 2. Materials and methods

### 2.1. General

The procedures used in these studies were approved by the West Virginia University Animal Care and Use

Committee (ACUC # 13-1101). All animals were older than the age normally associated with attainment of puberty for the breed/type and possessed a BCS of  $\geq 3$ .

Three studies were conducted to evaluate the effect of gonadotropic stimulation on the reproductive performance of nulliparous females. The first study utilized ewe lambs of mixed breeding ( $N=191$ ) born in mid-October through mid-December of 2010 and 2011 and was conducted during the mid-anestrus period (May to early July) of 2011 and 2012 on five farms located in West Virginia and southwestern Pennsylvania. The second and third studies were conducted beginning in May (study 2) and late August (study 3) at a farm located in southwestern Pennsylvania. The animals used in these studies consisted of spring-born Dorset X Texel yearlings ( $N=120$ , 15-months-old and  $N=104$ , 17-months-old for studies 2 and 3, respectively). In the third study a single blood sample was collected via jugular venipuncture and assayed for progesterone as described by Sheffel et al. (1982). The limit of detection was 0.1 ng/mL, and the intra and interassay coefficients of variation were 7.1 and 18%, respectively. Animals with concentrations of progesterone  $>1$  ng/mL ( $N=11$ ) were considered to be ovulatory and were removed from the study. Animals were not previously exposed to rams and were managed on mixed grass:legume pastures with ad libitum access to water and shade.

### 2.2. Treatments

To evaluate the effect of gonadotropic stimulation on fertility of fall-born ewe lambs (study 1) and yearlings (study 2) during the mid-anestrus period (May through July), a controlled internal drug releasing (CIDR) device (containing 0.3 g of progesterone; Pfizer Animal Health, New York, NY, now Zoetis Animal health, Kalamazoo, MI) was applied to each ewe for 5 days prior to ram introduction. At CIDR removal, ewes were assigned randomly to receive either gonadotropic stimulation with an injection of the gonadotropin mixture [3 mL P.G. 600®, i.m. (240 IU eCG, 120 IU hCG), Intervet, Millsboro, DE; CIDRPG] or no further treatment (CIDR) and were joined with a group of sexually mature rams at a ratio not less than 1 ram per 18 nulliparous ewes.

Study 3 was designed to evaluate the effect of gonadotropic stimulation on yearling nulliparous ewes during late anestrus or early breeding season. Treatments were similar to the first 2 studies, but also included a group of ewes that did not receive either progesterone or gonadotropic stimulation (Control).

### 2.3. Estrous detection

At each farm, rams fitted with marking harnesses were joined with ewes and ran as a single breeding group for approximately 30 (May–July, studies 1 and 2) or 75 days (August, study 3) beginning at CIDR removal. To detect estrus at the first service period, ewes were observed for the presence of raddle marks between 24 and 96 h after ram introduction.

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