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# 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-anestrous 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-anestrous period, but did not affect reproductive outcome in yearling females.



## 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 anestrous 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 anestrous ewes (Knights et al., 2001a,b). Attempts to induce fertile mating during anestrous 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 anestrous 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-anestrous 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 ≥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-anestrous 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-anestrous 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 anestrous 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.

**Table 1**
Effect of gonadotropic stimulation with 240 IU of eCG and 120 IU of hCG (P.G. 600®) administered on the last day of a 5-day progesterone pre-treatment on reproductive responses in fall-born ewe lambs exposed to rams during the mid-anestrus (study 1).

| Variable | Treatment | | *P*-value |
|---|---|---|---|
| | CIDR | CIDR + gonadotropin | |
| *n* | 95 | 95 | |
| Estrous response (%) | 52.6 ± 4.7 (*n* = 95) | 77.9 ± 4.7 (*n* = 95) | 0.0002 |
| Conception rate (%) | 78 ± 5.5 (*n* = 50) | 83.7 ± 4.5 (*n* = 74) | NS |
| Pregnancy rate (%) 1st service[a] | 41.1 ± 5.0 (*n* = 95) | 65.3 ± 5.0 (*n* = 95) | 0.0007 |
| Prolificacy first service[b] | 1.31 ± 0.1 (*n* = 36) | 1.5 ± 0.08 (*n* = 51) | NS |
| Overall prolificacy | 1.32 ± 0.1 (*n* = 38) | 1.47 ± 0.08 (*n* = 53) | NS |
| Lambing to 1st service (%)[c] | 37.9 ± 5.1 (*n* = 95) | 53.7 ± 5.1 (*n* = 95) | 0.03 |
| Lambing to 2nd service (%)[d] | 4.2 ± 1.8 (*n* = 95) | 2.1 ± 1.8 (*n* = 95) | NS |
| Pregnancy retention 1st service (%)[e] | 92.3 ± 5.2 (*n* = 39) | 85.5 ± 4.1 (*n* = 62) | NS |
| Proportion of females lambing (%) | 40.0 ± 5.1 (*n* = 95) | 55.8 ± 5.1 (*n* = 95) | 0.03 |
| Lambing rate (%)[f] | 52.6 ± 8.3 (*n* = 95) | 82 ± 8.2 (*n* = 95) | 0.01 |
| Ram introduction to lambing (d)[g] | 148.6 ± 0.5 (*n* = 38) | 147.3 ± 0.4 (*n* = 53) | 0.05 |
| Lambing day (d)[h] | 8.4 ± 0.5 (*n* = 38) | 6.8 ± 0.4 (*n* = 53) | 0.02 |

Not all variables were measured for all animals. n's are indicated for each variable under treatment groups.
[a] Number of ewes diagnosed pregnant on day 30–35 as a percentage of all ewes exposed to rams.
[b] Lambs born per ewe lambing to the first service period (first 14 days of lambing season.).
[c] Proportion of ewes lambing by day 14 of the lambing period.
[d] Proportion of ewes lambing between day 15 and day 30 of the lambing period.
[e] Ewes lambing that were diagnosed pregnant to the first service period.
[f] Lambs born per ewe exposed to rams.
[g] Mean number of days from ram introduction to lambing.
[h] Mean day ewe gave birth within the lambing period (day 1 = day the first ewe lambed). Values are least squares means ± SEM (number of animals).

### 2.4. Pregnancy diagnosis and lambing data

Pregnancy diagnosis was conducted using transrectal ultrasonography (Aloka 500′ Corometrics Medical Systems, Wallingford, CT, USA) with a 7.5-mHz, linear trans-rectal probe between 30–35 and 50–55 days after ram introduction to detect pregnancy conceived at the first and second service periods, respectively. In study 3, additional pregnancy diagnoses were conducted at 75 and 90 days post ram introduction. Lambing records were collected on all animals except five (5) that died during the experiment (Study 2, 2 CIDR and 1 CIDRPG; Study 3, 1 CIDR and 1 CIDRPG).

### 2.5. Statistical analysis

Analysis of variance (ANOVA) was conducted using the GLM procedure of SAS (Statistical Analysis System version 9.4 for Windows; SAS Institute, Cary, NC, USA) to determine effects of gonadotropic stimulation containing 240 IU of eCG and 120 IU of hCG in studies 1 and 2. The model consisted of the fixed effects of gonadotropic stimulation and farm. The farm X gonadotropic stimulation was included in study 1. In study 3, orthogonal contrasts were used to compare progesterone-treated vs none (CIDR and CIDRPG vs Control) and gonadotropin stimulation vs none in progesterone pre-treated animals (CIDR vs CIDRPG). The response variables included prolificacy (number of lambs born per ewe lambing), interval from ram introduction to lambing, mean lambing day (day that ewe lambed during the lambing period) and lambing rates (lambs born per ewe exposed).

The following traits were evaluated by Chi-square using the PROC FREQ procedure of SAS, proportion of ewes that showed estrus; pregnancy and conception rates (number of ewes pregnant at first pregnancy diagnosis as a percentage all ewes exposed to rams and of all ewes detected in estrus, respectively); proportion of ewes lambing (ewes lambing of all ewes treated); Proportion of ewes lambing to first, second or later service periods (proportion of ewes lambing by day 14, day 15 to day 30 or after day 30 of the lambing period (study 3 only), respectively); and pregnancy retention to the first and second services (ewes that lambed that were detected pregnant at the first and second pregnancy diagnosis, respectively).

## 3. Results

### 3.1. Study 1

In fall-born ewe lambs bred during the anestrous period (Table 1), gonadotropic stimulation increased estrous response ($P = 0.0002$), pregnancy rate to the first service period ($P = 0.0007$), proportion of ewes lambing to the first service period, proportion lambing overall ($P = 0.03$) and the lambing rate ($P = 0.01$). Treatment decreased the number of days from ram introduction to lambing ($P = 0.05$) and the mean lambing day ($P = 0.02$). The mean conception rate ($81.5 \pm 3.5\%$) and the proportion of fall-born ewe lambs lambing to the second service period ($3.2 \pm 1.3\%$) were not affected by gonadotropin stimulation.

### 3.2. Study 2

In study 2 gonadotropic stimulation increased estrous response ($P = 0.03$; Table 2) but did not modify other measures of reproductive performance in yearling females exposed to rams during seasonal anestrus.

**Table 2**
Effect of gonadotropic stimulation with 240 IU of eCG and 120 IU of hCG (P.G. 600®) administered on the last day of a 5-day progesterone pre-treatment on reproductive responses in yearling nulliparous ewes exposed to rams during the mid-anestrus (study 2).

| Variable | Treatment | | *P*-value |
|---|---|---|---|
| | CIDR | CIDR + gonadotropin | |
| *n* | 61 | 59 | |
| Age (days) | 446.9 ± 6 | 433.6 ± 5.8 | |
| Estrous response (%) | 80.3 ± 4.3 | 93.2 ± 4.3 | 0.03 |
| Conception rate (%) | 69.4 ± 6.6 | 70.9 ± 6.3 | NS |
| Pregnancy rate (%) 1st service[a] | 55.7 ± 6.2 | 66.1 ± 6.3 | NS |
| Prolificacy 1st service[b] | 1.46 ± 0.1 | 1.48 ± 0.1 | NS |
| Overall prolificacy | 1.38 ± 0.1 | 1.47 ± 0.09 | NS |
| Lambing to 1st service (%)[c] | 42.4 ± 6.5 | 43.1 ± 6.6 | NS |
| Lambing to 2nd service (%)[d] | 8.2 ± 3.6 | 8.5 ± 3.6 | NS |
| Pregnancy retention 1st service (%)[e] | 73.5 ± 8 | 65.8 ± 7.6 | NS |
| Proportion of females lambing (%) | 50.8 ± 6.6 | 51.7 ± 6.6 | NS |
| Lambing rate (%)[f] | 70 ± 10.7 | 75.9 ± 10.7 | NS |
| Ram introduction to lambing (d)[g] | 147.1 ± 1.17 | 147.1 ± 1.17 | NS |
| Lambing day (d)[h] | 9.1 ± 1.17 | 9.1 ± 1.17 | NS |

[a] Number of ewes diagnosed pregnant on day 33 as a percentage of all ewes exposed to rams.
[b] Lambs born per ewe lambing to the first service period (first 14 days of lambing season).
[c] Proportion of ewes lambing by day 14 of the lambing period.
[d] Proportion of ewes lambing between day 15 to day 30 of the lambing period.
[e] Ewes lambing that were diagnosed pregnant to the first service period.
[f] Lambs born per ewe exposed to rams.
[g] Mean number of days from ram introduction to lambing.
[h] Mean day ewe gave birth within the lambing period (day 1 = day the first ewe lambed). Values are least squares means ± SEM.

### 3.3. Study 3

During the transition into the breeding season, progesterone pre-treatment increased the proportion of yearling females exhibiting estrus during the first 3 days after ram introduction ($P = 0.0001$), pregnancy rate and proportion of females lambing to the first service period ($P = 0.003$ and $P = 0.02$, respectively; Table 3). The proportion of females lambing to the second service period was lower in progesterone-pretreated yearlings ($P = 0.05$; Table 3). Gonadotropic stimulation of progesterone pre-treated yearlings decreased the proportion of females lambing to

**Table 3**
Effect of progesterone pre-treatment and gonadotropin stimulation with 240 IU of eCG and 120 IU of hCG (P.G. 600®) administered at progesterone removal on reproductive responses in spring-born yearling ewes exposed to rams during late anestrus/early breeding season (study 3).

| Variable | Treatment | | | *P*-value | |
|---|---|---|---|---|---|
| | Control | CIDR | CIDR + gonadotropin | Control vs CIDR | CIDR + gonadotropin vs CIDR |
| *n* | 30 | 31 | 32 | | |
| Age | 509 ± 8.9 | 512 ± 9.1 | 510 ± 8.6 | NS | NS |
| Estrous response (%) | 16.6 ± 8.1 | 61.3 ± 8.0 | 71.9 ± 7.8 | 0.0001 | NS |
| Conception rate (%) | 50.0 ± 25.8 | 52.9 ± 12.5 | 47.8 ± 10.8 | NS | NS |
| Pregnancy rate (%) 1st service[a] | 10.0 ± 8.1 | 45.2 ± 8.0 | 34.4 ± 7.8 | 0.003 | NS |
| Pregnancy rate (%) 2nd service [b] | 44.8 ± 9.3 | 58.1 ± 9.0 | 43.8 ± 8.9 | NS | NS |
| Pregnancy rate (%) 3rd service[c] | 72.4 ± 8.4 | 71 ± 8.1 | 74.2 ± 8.1 | NS | NS |
| Pregnancy rate (%) 4th service[d] | 80.0 ± 7.4 | 87.1 ± 7.3 | 71 ± 7.3 | NS | NS |
| Prolificacy 1st service[e] | 1.50 ± 0.3 | 1.2 ± 0.1 | 1.6 ± 0.2 | NS | NS |
| Overall Prolificacy | 1.52 ± 0.1 | 1.35 ± 0.1 | 1.33 ± 0.1 | NS | NS |
| Lambing to 1st service (%)[f] | 6.9 ± 7.2 | 38.7 ± 7.0 | 18.1 ± 7.0 | 0.02 | 0.02 |
| Lambing to 2nd service (%)[g] | 34.5 ± 7.7 | 16.1 ± 7.4 | 18.1 ± 7.4 | 0.05 | NS |
| Lambing to 3rd service (%)[h] | 40 ± 8.4 | 25.8 ± 8.2 | 25 ± 8.2 | NS | NS |
| Proportion of females lambing (%) | 76.7 ± 7.6 | 86.7 ± 7.6 | 66.7 ± 8.1 | NS | 0.08 |
| Lambing rate (%)[i] | 101 ± 13.6 | 113 ± 13.6 | 88.9 ± 14.3 | NS | NS |
| Ram introduction to lambing (d)[j] | 178 ± 4.5 | 172 ± 4.2 | 170 ± 5.2 | NS | NS |
| Lambing day (d)[k] | 33 ± 4.5 | 25.1 ± 4.3 | 24.6 ± 5.2 | NS | NS |

[a–d] Number of ewes diagnosed pregnant on day 35, 50, 75 and 90 respectively, as a percentage of all ewes exposed to rams.
[e] Lambs born per ewe lambing to the first service period (first 14 days of lambing season).
[f] Proportion of ewes lambing by day 14 of the lambing period.
[g] Proportion of ewes lambing between day 15 and 30 of the lambing period.
[h] Proportion of ewes lambing after day 30 of the lambing period.
[i] Lambs born per ewe exposed to rams.
[j] Mean number of days from ram introduction to lambing.
[k] Mean day ewe gave birth within the lambing period (day 1 = day the first ewe lambed). Values are least squares means ± SEM.

the first service period ($P = 0.02$; Table 3) but did not modify other reproductive performance variables measured.

## 4. Discussion

Successful breeding of fall-born ewe lambs or spring-born yearlings so as to reduce the age at first lambing requires that they are bred during the seasonal anestrous period. Pre-treatment of adult non-lactating ewes during the seasonal anestrous period with progesterone for 5 or 12 days followed by ram introduction induced fertile estrus and lambing in 65–80% of ewes (Knights et al., 2001a,b; 2003; D'Souza et al., 2014). In contrast, yearlings pre-treated with progesterone alone prior to introduction of rams failed to exhibit estrus (Burfening and Van Horn, 1970). In the present study gonadotropic stimulation of progesterone-pretreated fall born ewe lambs and yearlings increased estrous response, pregnancy rate to first service and lambing rate by approximately 20%. However, there was no effect of treatments on conception rate or prolificacy. This indicates that the major effect of the gonadotropic stimulation was to increase the proportion of ewe lambs that were mated.

Induction of estrus during the anestrous period is dependent on the "ram-effect" response in ewes which includes increased gonadotropin secretion, follicular growth and increased production of estrogen (Atkinson and Williamson, 1985; Oldham et al., 1978). Introduction of rams induced the ram-effect in progesterone-pre-treated 8-month old, fall-born ewe lambs during seasonal anestrus, but only 45% of ewe lambs expressed estrus (Knights et al., 2002). However, when progestogen pre-treated fall-born ewe lambs or yearlings were treated with estrogen (Knights et al., 2002) or eCG (Burfening and Van Horn, 1970) at the time of progestogen removal, most nulliparous females exhibited estrus. Production of estrogen in response to treatment with eCG was greater in ewe lambs than in mature ewes during the breeding season (Quirke et al., 1981) indicating that the ovarian follicles of ewe lambs are sensitive to gonadotropic stimulation. Whether there is a decrease sensitivity of follicles to gonadotropins or a reduced sensitivity of behavioral centers to estrogen in nulliparous females during anestrous is yet to be determined. However, ram-induced gonadotropin secretion might not stimulate production of sufficient quantities of estrogen to induce estrus in most ewe lambs during seasonal anestrus. Supplementation with estrogen directly (Knights et al., 2002) or with gonadotropins (Ainsworth and Shrestha, 1987; Ainsworth et al., 1991 and current study) improved reproductive performance in fall-born ewe lambs exposed to rams during mid-anestrous. Gonadotropic supplementation of yearlings bred during seasonal anestrus may be necessary (Robinson and Smith, 1967; Burfening and Van Horn, 1970) or of little benefit (current study) depending on the extent to which the ram effect response is maximized.

Gonadotropic supplementation improved reproductive performance in fall-born ewe lambs (current study) and was used in combination with progestogen pre-treatment to induce fertile estrus in yearlings (Robinson and Smith, 1967; Burfening and Van Horn, 1970). However, gonadotropic stimulation did not improve overall reproductive performance in progesterone-pretreated yearlings in the current study and had little or no effect on overall reproductive performance in non-lactating mature ewes treated with FSH (Knights et al., 2001a,b) or with the gonadotropin mixture, containing 240IU of eCG and 120IU of hCG (D'Souza et al., 2014). Further, in cross-bred sheep selected for lambing in fall, 59% of adult ewes were successfully bred in May, but only 11% of ewe lambs displayed estrus during the seasonal anestrous period (al-Shorepy and Notter, 1996). These findings indicate that the need for gonadotropic supplementation might wane with age and parity.

In the present study, progesterone pre-treatment of yearling acyclic ewes during the transition to the breeding season induced fertile estrus and advanced the breeding and lambing season. Gonadotropic supplementation did not enhance reproductive performance of the progesterone-treated females, which indicated that the need for gonadotropin stimulation might diminish with advancement into the breeding season. In contrast, gonadotropic stimulation following progestogen treatment advanced the breeding and lambing season of spring-born ewe lambs exposed to rams during the breeding season at 7 months of age (Stellflug et al., 2001; Sawalha et al., 2011). However, these studies did not include a progestogen-only treatment group which precluded an assessment of the requirement for gonadotropic stimulation beyond the progesterone pre-treatment.

The values for pregnancy rate to first service, proportion of ewes lambing to the first service period and prolificacy for gonadotropin-stimulated nulliparous ewes bred during anestrus in this study were similar to values reported previously for mature ewes (Knights et al., 2001a,b; D'Souza et al., 2014). However, the overall proportion of ewes lambing and lambing rate were lower for the nulliparous ewes than those observed in gonadotropin-stimulated and unstimulated adult females (D'Souza et al., 2014). In previous studies 20% of adult ewes conceived and lambed to the second service period (Knights et al., 2001a,b; D'Souza et al., 2014). Only 4% of fall-born ewe lambs and 8% of yearlings lambed to the second service period, resulting in lower overall fertility of the nulliparous ewes compared to that reported for adult ewes bred during mid-anestrous. In contrast, most yearlings bred during late anestrus conceived and lambed to the second or subsequent service periods. The low proportion of nulliparous ewes conceiving to the second mating opportunity during mid-anestrus might be related to reversion to an anestrous state following the first ram-induced ovulation (Oldham and Fisher, 1992), while higher pregnancy rate and lambing percentage of yearlings bred during late anestrous reflects an increase in fertility with progression into the breeding season.

Higher doses of eCG (500 IU) than used in this study resulted in improved reproductive outcome in mature ewes bred to artificial insemination (Langford et al., 1983). However, production of estrogen in response to treatment with eCG is greater in ewe lambs than in mature ewes (Quirke et al., 1981) and doses as low as 200–250 IU eCG was sufficient to improve reproductive performance

in mature ewes, while a 750 IU eCG decreased fertility (Gordon, 1971; Oldham and Pearce, 1984). No difference in reproductive performance was observed in ewes treated with 250 or 500 IU eCG (Ainsworth and Shrestha, 1985), or in ewe lambs treated with 200 or 400 IU eCG (Rekik et al., 2002). Additionally, a gonadotropin mixture of 400 IU eCG and 200 IU hCG (Safranski et al., 1992; Jabbar et al., 1994; Windorski et al., 2008) did not improve fertility and resulted in lambing rates comparable to or lower than that observed in the current study. These data leads to the suggestion that increasing the dosage of gonadotropin supplementation does not always result in further improvements in reproductive outcomes and that a lower dose of eCG might be optimal for nulliparous females.

Induction of the "male effect" and fertile estrus in ewes bred during anestrus is dependent on exposure to healthy, fertile rams, at an appropriate ram: ewe ratio. In the present study a ram: ewe ratio of 1:18 was used, which is similar to the ram: ewe ratios used to induce fertile estrus in mature dry ewes (Jabbar et al., 1994; Knights et al., 2001a,b; D'Souza et al., 2014). The relatively high estrous response observed in females pre-treated with progesterone only in this study, suggest that a strong male effect response was induced. Scrotal circumference, semen quality and reproductive performance of rams decline during seasonal anestrous but these variables can be improved by pre-treatment with melatonin or manipulation of light exposure (Palacín et al., 2008). However, as semen characteristics were not evaluated and rams were not pre-treated to improve libido and semen quality, the overall lower fertility observed in the nulliparous females in this study might be related to lower fertility in rams. Further, as specific ram: ewe ratios have not been established for nulliparous females, it is possible that a higher ram: ewe ratio than used in this study might improve conception rates overall, and increase the proportion of females bred at the second service period during anestrous.

Pregnancy retention was not affected by treatments and varied between 65 and 92% across all experiments. Pregnancy retention as low as 70% have been reported for adult ewes bred out-of-season (Knights et al., 2001a,b; Dixon et al., 2007). Embryonic and fetal survival is lower in younger and lighter females (Shorten et al., 2013), which might impart explain the low pregnancy retention and the overall lower lambing rate observed in the nulliparous females in this study.

In conclusion, gonadotropic stimulation with a gonadotropin mixture containing 240 IU of eCG and 120 IU of hCG following progesterone pre-treatment improved reproductive outcome in fall-born ewe lambs bred during the mid-anestrous period with estrous response and pregnancy rate to the first service period comparable to that observed in adult females. Progesterone pre-treatment alone was sufficient to induce fertile estrus and advance the breeding season in yearlings bred during mid- and late-anestrus. However, delaying breeding until later in the breeding season should be considered as the potential benefit associated with advancing the breeding season might not offset the cost associated with progesterone pre-treatment.

## Conflict of interest

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## References

Ainsworth, L., Shrestha, J.N.B., 1985. Effect of PMSG dosage on the reproductive performance of adult ewes and ewe lambs bred at a progestogen-PMSG synchronized estrus. Theriogenology 24, 479–487.

Ainsworth, L., Shrestha, J.N.B., 1987. The reproductive performance of ewe lambs in a controlled environment. Anim. Prod. 44, 233–240.

Ainsworth, L., Heaney, D.P., Shrestha, J.N.B., 1991. Age at puberty, fertility and litter size of ewe lambs reared under different photoregimens. Theriogenology 36, 401–409.

al-Shorepy, S.A., Notter, D.R., 1996. Genetic variation and covariation for ewe reproduction, lamb growth, and lamb scrotal circumference in a fall-lambing sheep flock. J. Anim. Sci. 74, 1490–1498.

Atkinson, S., Williamson, P., 1985. Ram-induced growth of ovarian follicles and gonadotrophin inhibition in anoestrous ewes. J. Reprod. Fertil. 73, 185–189.

Burfening, P.J., Van Horn, J.L., 1970. Induction of fertile oestrus in prepubertal ewes during the anoestrous season. J. Reprod. Fertil. 23, 147–150.

Dixon, A.B., Knights, M., Winkler, J.L., Marsh, D.J., Pate, J.L., Wilson, M.E., Dailey, R.A., Inskeep, E.K., 2007. Patterns of late embryonic and fetal mortality and association with several factors in sheep. J. Anim. Sci. 85, 1274–1284.

D'Souza, K.N., Rastle-Simpson, S.L., Redhead, A.K., Baptiste, Q.S., Smith, B., Knights, M., 2014. Gonadotropin stimulation using P.G. 600® on reproductive success of non-lactating anestrous ewes. Anim. Reprod. Sci. 148, 115–120.

Forcada, F., Abecia, J.A., Zarazaga, L., 1991. A note on attainment of puberty of September-born early-maturing ewe lambs in relation to level of nutrition. Anim. Sci. 53, 407–409.

Gaskins, C.T., Snowder, G.D., Westman, M.K., Evans, M., 2005. Influence of body weight, age, and weight gain on fertility and prolificacy in four breeds of ewe lambs. J. Anim. Sci. 83, 1680–1689.

Gordon, I., 1971. Induction of early breeding in sheep by standard and modified progestagen-PMS treatments. J. Agric. Sci. 76, 337–341.

Hafez, E.S.E., 1952. Reproduction of the ewe. J. Agric. Sci. 42, 189–265.

Hohenboken, W., Vavra, M., Phillips, R., McArthur, J.A.B., 1977. The effects of age at first lambing on production and longevity of Columbia and Targhee ewes. In: Station, O.A.E. (Ed.), Technical Bulletin. Oregon State University, Corvallis.

Jabbar, G., Umberger, S.H., Lewis, G.S., 1994. Melengesterol acetate and norgestomet for the induction of synchronized estrus in seasonally anovular ewes. J. Anim. Sci. 72, 3049–3054.

Knights, M., Baptiste, Q.S., Dixon, A.B., Pate, J.L., Marsh, D., Inskeep, E.K., Lewis, P.E., 2003. Effect of dosage of follicle stimulating hormone (FSH), vehicle and time of injection on ovulation rate and prolificacy in anestrous ewes. Small Rumin. Res. 50, 1–9.

Knights, M., Baptiste, Q.S., Lewis, P.E., 2002. Ability of ram introduction to induce LH secretion, estrus and ovulation in fall-born ewe lambs during anestrus. Anim. Reprod. Sci. 69, 199–209.

Knights, M., Hoehn, T., Lewis, P.E., Inskeep, E.K., 2001a. Effectiveness of intravaginal progesterone inserts and FSH for inducing synchronized estrus and increasing lambing rate in anestrous ewes. J. Anim. Sci. 79, 1120–1131.

Knights, M., Maze, T.D., Bridges, P.J., Lewis, P.E., Inskeep, E.K., 2001b. Short-term treatment with a controlled internal drug releasing

(CIDR) device and FSH to induce fertile estrus and increase prolificacy in anestrous ewes. Theriogenology 55, 1181–1191.
Langford, G.A., Marcus, G.J., Batra, T.R., 1983. Seasonal effects of PMSG and number of inseminations on fertility of progestogen-treated sheep. J. Anim. Sci. 57, 307–312.
López-Sebastian, A., Gómez-Brunet, A., Pinilla, A., Cabrillos, B., 1985. Influencia de la epoca de nacimiento Y el effecto de los machos sobre la aparicion de la pubertad en corderas manchegas. ITEA 58, 45–50.
Oldham, C.M., Fisher, J., 1992. Utilizing the ram effect. In: Proceedings of the Out of Season Breeding Symposium, Iowa State University, pp. 33–54.
Oldham, C.M., Pearce, D.T., 1984. The sensitivity of ovarian and oestrous activity in Southdown ewes to an intramuscular injection of pregnant mare serum gonadotropin. Anim. Prod. 1 (5), 728 (in Australia).
Oldham, C.M., Martin, G.B., Knight, T.W., 1978. Stimulation of seasonally anovular Merino ewes by rams. Part I. Time from introduction of rams to the preovulatory surge and ovulation. Anim. Reprod. Sci. 1, 283–290.
Palacín, I., Abecia, J.A., Forcada, F., Casao, A., Cebrian-Perez, J.A., Muino-Blanco, T., Palacios, C., Pontes, J.M., 2008. Effect of exogenous melatonin treatment on out of season ram fertility. Ital. J. Anim. Sci. 7, 199–206.
Quirke, J.F., 1978. Reproductive performance of Galway, Finnish Landrace, and Finncross ewe lambs. Ir. J. Agric. Res., 25–32.
Quirke, J.F., Hanrahan, J.P., Gosling, J.P., 1981. Duration of oestrus, ovulation rate, time of ovulation and plasma LH, total oestrogen and progesterone in Galway adult ewes and ewe lambs. J. Reprod. Fertil. 61, 265–272.
Quirke, J.F., Stabenfeldt, G.H., Bradford, G.E., 1985. Onset of puberty and duration of the breeding season in Suffolk, Rambouillet, Finnish Landrace, Dorset and Finn-Dorset ewe lambs. J. Anim. Sci. 60, 1463–1471.
Rekik, M., Lassouued, N., Yacoubi, C., 2002. Reproductive performances in ewe lambs of the Queue Fine de l'Ouest breed and their D'man crosses following synchronization. Small Rumin. Res. 45, 75–78.
Robinson, T.J., Smith, J.F., 1967. The evaluation of SC-9880-impregnated intravaginal sponges used with or without PMSG for the advancement of the breeding season of British ewes. In: Robinson, T.J. (Ed.), The Control of the Ovarian Cycle in the Sheep. Sydney University Press, Sydney.
Safranski, T.J., Lamberson, W.R., Keisler, D.H., 1992. Use of melengestrol acetate and gonadotropins to induce fertile estrus in seasonally anestrous ewes. J. Anim. Sci. 70, 2935–2941.
Sawalha, M.N., Kridli, R.T., Jawasreh, K.I., Meza-Herrera, C.A., 2011. The use of melatonin and progestagen – eCG to initiate reproductive activity in prepuberal Awassi ewe lambs. Trop. Anim. Health Prod. 43, 1345–1350.
Sheffel, C.E., Pratt, B.R., Ferrell, W.L., Inskeep, E.K., 1982. Induced corpora lutea in the postpartum beef cow. II. Effects of treatment with progestogen and gonadotropins. J. Anim. Sci. 54, 830–836.
Shorten, P.R., O'Connell, A.R., Demmers, K.J., Edwards, S.J., Cullen, N.G., Juengel, J.L., 2013. Effect of age, weight, and sire on embryo and fetal survival in sheep. J. Anim. Sci. 91, 4641–4653.
Spencer, D.A., Schott, R.G., Phillips, R.W., Aune, B., 1942. Performance of ewes bred first as lambs compared with ewes bred first as yearlings. J. Anim. Sci. 1, 27–33.
Stellflug, J.N., Rodriguez, F., Fitzgerald, J.A., 1993. Influence of estrus induction with artificial insemination or natural mating on reproductive performance of fall-born ewe lambs during an out-of-season breeding. Sheep Res. J. 9, 115–118.
Stellflug, J.N., Hatfield, P.G., Wulster-Radcliffe, M.C., Walker, J.W., 2001. Reproductive performance of ewe lambs from ewes from different selection practices with or without induced estrus. Anim. Reprod. Sci. 66, 185–193.
Vesely, J.A., Swierstra, E.E., 1987. Reproductive traits of ewe lambs representing eight genetic types born in winter, spring, summer and fall. J. Anim. Sci. 65, 1195–1200.
Windorski, E.J., Schauer, C.S., Wurst, A.K., Inskeep, E.K., Luther, J.S., 2008. Effects of melengesterol acetate and P.G.600 on fertility in Ramnbouillet ewes outside the natural breeding season. Theriogenology 70, 227–232.