The Effect of Ram Exposure on Uterine Involution and Luteal Function During the Postpartum Period of Hair Sheep Ewes in the Tropics[1]

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ABSTRACT: St. Croix White hair sheep ewes lambing in July (n = 20) or November (n = 26) were used to evaluate the effect of ram exposure on uterine involution and postpartum luteal function. Ewes were exposed to an epididymectomized ram (EXPOSED) beginning on d 7 after lambing (d 0) or kept isolated from rams (CONTROL) through d 63. The width of each uterine horn was measured using transrectal ultrasonography at 3.5-d intervals beginning within 3 d after lambing. Jugular blood samples were also collected at these times, and plasma was harvested for progesterone (P_4) analysis. Days to first estrus postpartum was not different ($P > .10$) between EXPOSED ewes that lambed in July or November (39.3 ± 3.1 vs 44.2 ± 3.8 d, respectively). Cross-sectional area of uterine horns was not different ($P > .10$) between EXPOSED and CONTROL ewes, ewes bearing one or two lambs, or ewes that lambed in November or July. Cross-sectional area of uterine horns in EXPOSED and CONTROL ewes had decreased to < 30% of initial values by 28 d postpartum ($P < .0001$). Ewes exposed to rams had a P_4 concentration greater than 1 ng/mL sooner postpartum ($P < .006$) than CONTROL ewes (32.4 ± 2.4 vs 42.1 ± 2.3 d, respectively). The P_4 concentration in the first sample greater than 1 ng/mL was greater ($P < .06$) in EXPOSED ewes than in CONTROL ewes (3.3 ± .4 vs 2.3 ± .4 ng/mL, respectively). In July, ewes exposed to rams had greater ($P < .03$) P_4 concentrations than CONTROL ewes during the 63 d after parturition, but this difference was not apparent ($P > .10$) in ewes that lambed in November. Ram exposure did not hasten uterine involution in hair sheep ewes in the tropics. Luteal function, determined by plasma P_4 concentrations, was enhanced by ram exposure during July but not during November. The lack of seasonality of hair sheep in the tropics does not seem to totally inhibit the response of ewes to ram exposure.

Key Words: Sheep, Postpartum Period, Progesterone, Uterus

 J. Anim. Sci. 1998. 76:3090–3094

Introduction

During the early postpartum period, physical and physiological conditions exist that prevent pregnancy. During gestation, the uterus is stretched to several times its size by the developing fetus and the placental structures. After parturition, the uterus decreases in size prior to the resumption of normal estrous cycles and fertility. The involuting uterus has been described as a temporary barrier to fertility during the early postpartum period (Kiracofe, 1980). Even if ovulation occurred and fertilization took place, the embryo would have very little chance of implanting in the uterus. Kiracofe (1980) cites data from several studies that uterine involution is complete by 25 d postpartum in wool ewes in temperate climates.

The ram effect has been shown to be useful for inducing estrous cyclicity in anestrous ewes (Wheaton et al., 1992; Lindsay, 1996). Lindsay (1996) has indicated that the presence of a ram can induce anestrous ewes to exhibit estrus and provide some degree of synchrony. Ewes in the U.S. Virgin Islands (**USVI**) do not exhibit a seasonal anestrous period (Evans et al., 1991), and work done in our laboratory with hair sheep ewes has shown that the presence of a ram does not induce synchronization of estrus in cyclic ewes (R. W. Godfrey, unpublished data). Hair sheep ewes in the USVI exhibit estrous cycles accompanied by ovulation during all times of the year (Wildeus et al., 1991). The only time that ewes do not exhibit estrous cycles is during gestation or the early postpartum period. This study evaluated the effect of ex-

[1]The authors would like to thank Victor Callas, Jr., for assistance with animal care and management. This study was a contribution to the North Central Regional Research Project NC-111, Increased Efficiency of Sheep Production.

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Received March 16, 1998.

Accepted August 13, 1998.

Table 1. Distribution of ewes raising single or twin lambs in July or November among ewes exposed to a ram (EXPOSED) or kept isolated from the ram (CONTROL) during the postpartum period

	Treatment group	
Month	CONTROL	EXPOSED
July		
Singles	5[a]	5
Twins	5	5
November		
Singles	5	3
Twins	9	9

[a]Number of ewes in each group.

posure to a ram during the postpartum anestrous period of hair sheep ewes in the tropics on the rate of uterine involution and the time to first estrus.

Materials and Methods

St. Croix White hair sheep ewes that lambed in either July (n = 20) or November (n = 26) were used. Ewes ranged in age from 2 to 6 yr. All ewes had been bred to St. Croix White rams during the previous breeding season. Ewes were assigned to treatment groups on the day of lambing (d 0) based on the number of lambs born (Table 1). One treatment group consisted of placing ewes (n = 10 in July and n = 12 in November) in a guinea grass (*Panicum maximum*) pasture (.8 ha) with an epididymectomized ram (**EXPOSED**) beginning on d 7. The ram was equipped with a marking harness to aid in detecting estrus. Ewes in the EXPOSED groups were checked daily for crayon marks from the ram. The remaining ewes (**CONTROL**; n = 10 in July and n = 14 in November) were kept in a guinea grass pasture (.9 ha) isolated from any contact with rams. Ewes remained in their respective treatment groups until lambs were weaned on d 63.

Beginning on d 3, the diameter of the left and right uterine horns was measured using transrectal ultrasonography every 3.5 d until weaning at d 63. Ewes were placed in dorsal recumbency in a tilting squeeze chute for the procedure. A 5-MHz, linear array transducer was used (Pie Medical Scanner, Scanner 450, Classic Medical Supply, Tequesta, FL). Images of each uterine horn were captured using a Sony Video Graphic Printer (model UP-870MD, Classic Medical Supply). Upon locating each uterine horn in the image on the screen, the image was frozen, and the diameter was measured using the caliper function. Cross-sectional area was calculated as area = $\pi \times (\text{diameter}/2)^2$.

On the days of uterine horn measurement, a jugular blood sample was taken from each ewe. Plasma was harvested and stored at –20°C until it was analyzed for progesterone (**P_4**) concentration with an ELISA. Plasma samples (10 μL) were assayed in duplicate according to the instructions that came with the ELISA kits (Ovusure, CDMV, Quebec, Canada). Absorbance values were determined using a microplate reader and were converted to concentration using SOFTmax software (Molecular Devices, Menlo Park, CA). The sensitivity of the assay was .5 ng/mL and the inter- and intraassay CV were < 10 and 5%, respectively.

Data were analyzed using the General Linear Models procedures of SAS (1996). Cross-sectional area of uterine horns and progesterone values were analyzed using repeated measures techniques with treatment (EXPOSED, CONTROL), days postpartum, time of year of lambing, and number of lambs born in the model. Postpartum interval to estrus could only be determined for ewes in the EXPOSED group and was analyzed using time of year of lambing and number of lambs as the main effects. The time to the first P_4 value greater than 1 ng/mL and the value of the first P_4 sample greater than 1 ng/mL were analyzed using ram exposure, time of year of lambing, number of lambs born, and treatment group in the model. All values are reported as least squares means ± SEM.

Results

There was no effect of time of year of lambing ($P > .10$) on the cross-sectional area of uterine horns during the postpartum period, so the data were pooled for final analysis. Cross-sectional area of the uterine horns was similar ($P > .10$) between ewes that gave birth to single or twin lambs (Figure 1a). There was no difference ($P > .10$) in the cross-sectional area of the uterine horns between the EXPOSED and CONTROL ewes (Figure 1b) during the 63-d postpartum period. Cross-sectional area of uterine horns in EXPOSED and CONTROL ewes had decreased to < 30% of initial values by 28 d postpartum ($P < .0001$) with no further measurable decrease over time.

Ewes that lambed in November and were exposed to rams had a similar ($P > .10$) P_4 concentration to ewes that were isolated from rams (Figure 2a). In contrast, ewes that lambed in July and were exposed to rams during the postpartum period had a higher ($P < .03$) P_4 concentration than CONTROL ewes (Figure 2b). One EXPOSED ewe failed to show estrus or a rise in P_4 by d 63, and three CONTROL ewes failed to show a rise in P_4 in November. In the July lambing ewes, two EXPOSED ewes failed to show estrus, and four CONTROL ewes failed to exhibit a rise in P_4 by d 63.

The time to estrus, which was only determined for the EXPOSED ewes, was not different ($P > .10$) between November and July lambing ewes (Table 2). There was no difference ($P > .10$) in the interval to

first estrus postpartum between EXPOSED ewes raising one lamb and ewes raising twins (48.3 ± 5.0 vs 37.7 ± 4.2 d, respectively). The number of days postpartum to a P_4 value greater than 1 ng/mL was less ($P < .006$) for EXPOSED than for CONTROL ewes, regardless of the time of year of lambing (Table 2). The concentration of P_4 in the first sample postpartum that was greater than 1 ng/mL was higher ($P < .06$) in the EXPOSED ewes that lambed in November than in CONTROL ewes that lambed in November (Table 2). There was no difference ($P > .10$) in the P_4 concentration of the first sample greater than 1 ng/mL between CONTROL and EXPOSED ewes that lambed in July. There was no interaction ($P > .10$) of time of year of lambing and treatment group on the first P_4 sample greater than 1 ng/mL, so the data were pooled and analyzed. The EXPOSED ewes had a higher ($P < .05$) P_4 concentration in the first sample that was greater than 1 ng/mL than CONTROL ewes (Table 2).

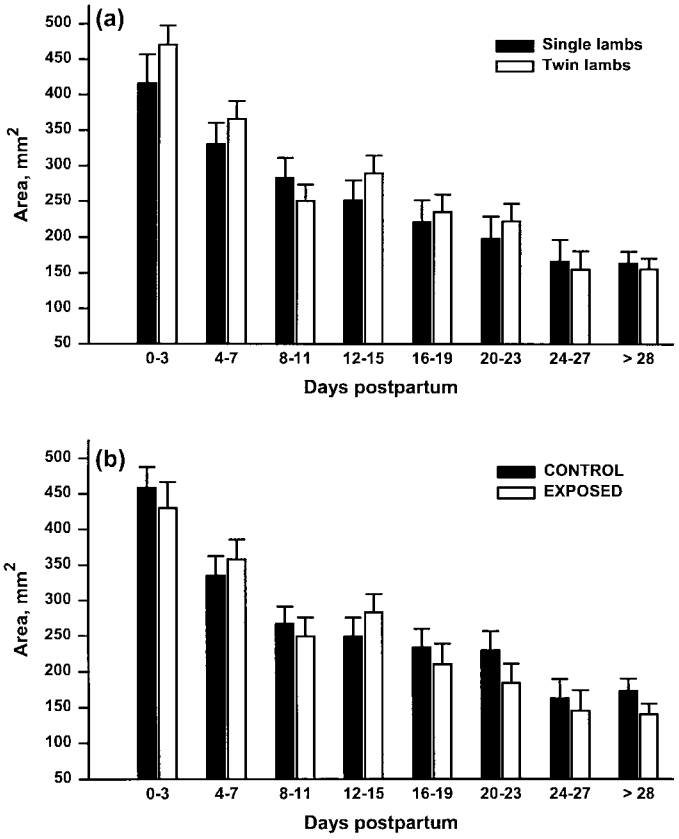

Figure 1. Cross-sectional area of uterine horns of hair sheep ewes that (a) gave birth to single (n = 18; solid bars) or twin (n = 28; open bars) lambs and (b) ewes that were isolated (n = 24; solid bars) from a ram during the postpartum period or exposed to a ram (n = 22; open bars) beginning at d 7 postpartum (lambing = d 0). There was no effect ($P > .10$) of either number of lambs born or ram exposure on the rate of uterine involution as determined by cross-sectional area.

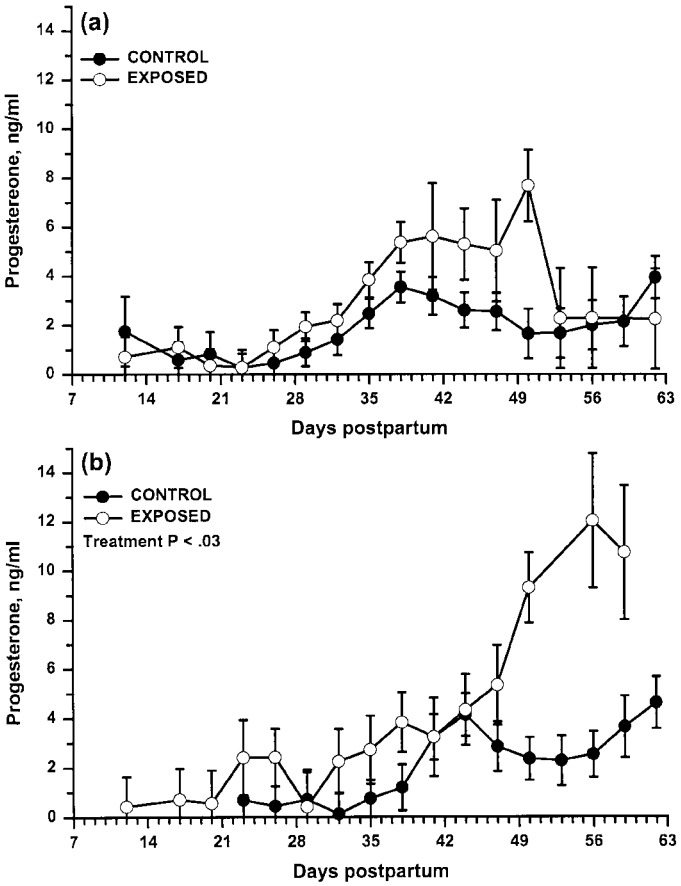

Figure 2. Progesterone profiles of ewes that lambed in (a) November or (b) July and were kept isolated from the ram (solid circles) for 63 d or exposed to a ram (open circles) beginning on d 7 postpartum (lambing = d 0). There was no effect ($P > .10$) of ram exposure on progesterone concentration in ewes lambing in November, but ewes lambing in July that were exposed to a ram had higher ($P < .03$) progesterone than control ewes.

Discussion

The time to completion of uterine involution varies in livestock species. Greyling and van Niekerk (1991) reported that the diameter of the uterine horns of Boer goats returned to the normal, nonpregnant size by 27.9 d postpartum. In Holstein cows, the uterus was not fully involuted until 41.5 d postpartum (Kamimura et al., 1993). Custer et al. (1990) found that the uterus of postpartum cows was involuted by 5 wk postpartum with or without exposure to a bull during this time. Kiracofe (1980) reported that uterine involution was complete by 3 wk postpartum in ewes, and by d 25 the uterus was similar in size to the nonpregnant uterus. This is in agreement with the data being presented; uterine involution was complete by d 28 postpartum. After d 28 there was no measurable decrease in cross-sectional area of the

Table 2. Progesterone (P_4) concentration (ng/mL) and time to estrus in ewes that lambed at different times of the year and were either exposed to a ram (EXPOSED) or kept isolated from the ram (CONTROL) during the postpartum period

Item	Days to estrus[a]	Days to P_4 > 1 ng/mL	P_4 at > 1 ng/mL[b]
November lambing ewes			
CONTROL	—	41.0 ± 2.9[c]	2.2 ± .5[e]
EXPOSED	44.2 ± 4.2	32.2 ± 3.1[d]	3.4 ± .5[f]
July lambing ewes			
CONTROL	—	43.5 ± 3.7[c]	2.6 ± .6
EXPOSED	39.3 ± 4.7	32.7 ± 3.9[d]	3.2 ± .6
Pooled			
CONTROL	—	42.1 ± 2.3[g]	2.3 ± .3[c]
EXPOSED	41.8 ± 3.1	32.4 ± 2.4[h]	3.3 ± .4[d]

[a]Days to estrus was not determined for CONTROL ewes due to the nature of the treatment.
[b]The first sample with a P_4 concentration > 1 ng/mL was used for this determination.
[c,d]Means within a column division with different superscripts are different ($P < .05$).
[e,f]Means within a column division with different superscripts are different ($P < .06$).
[g,h]Means within a column division with different superscripts are different ($P < .006$).

uterine horns in either the EXPOSED or CONTROL ewes.

The use of the ram effect to induce ewes to express estrous cycles has been shown to be effective only when the ewes are anestrous (Wheaton et al., 1992; Scott and Johnstone, 1994; Lindsay, 1996). Scott and Johnstone (1994) have shown that some variation in the response of ewes to the ram effect is determined by the depth of anestrus at the time the ewes are exposed to the ram. The ewes in the present study were exposed to the ram during the early postpartum period (d 7 after parturition), which is likely to be the only time the ewes in the USVI can be considered to be anestrous. Ewes in the EXPOSED groups exhibited estrus by 39 to 44 d postpartum, and, because of the design of the study, it was not possible to detect estrus in the CONTROL ewes. Based on concentrations of P_4, the EXPOSED ewes seem to have ovulated by d 32 postpartum (Table 2), but the first ovulation postpartum was not associated with estrus, because the first observed estrus occurred at 42 d postpartum. This difference of 10 d between first luteal activity and observed estrus indicates that the ewes exposed to rams in July or November apparently exhibited shortened luteal phases. The effect of the short luteal phase on subsequent estrous cycles or fertility could not be determined within the constraints of the study.

Evans et al. (1991) reported that St. Croix White hair sheep ewes in the USVI do not exhibit a seasonal pattern of estrous cycles. The ewes did exhibit a period of anestrus when they were moved to a higher latitude (Evans et al., 1991). Because of this lack of an anestrous period, the hair sheep flock at the University of the Virgin Islands is managed on an accelerated lambing schedule in which the ewes produce three lamb crops every 2 yr. Standard procedure is to keep the ewes and rams separate at all times except during the 35-d breeding period. In the present study, the ewes were exposed to rams at d 7 postpartum until lambs were weaned at d 63. The higher P_4 levels of the EXPOSED ewes in July compared to the EXPOSED ewes in November may indicate that the ewes were exhibiting some degree of seasonal response to ram exposure. Ewes at higher latitudes would be anestrous during July, which corresponds to the time of P_4 response to ram exposure in the present study. The day length on St. Croix is approximately 13.2 h in July and 11.2 h in November (Evans et al., 1991). This difference of 2 h may be just enough to allow the ewes to respond to the ram exposure during July, by having enhanced corpus luteum function, as evidenced by higher concentrations of P_4 postpartum.

In addition to the aforementioned difference in P_4 profile of EXPOSED ewes at different times of the year, EXPOSED ewes had P_4 levels greater than 1 ng/mL earlier in the postpartum period than CONTROL ewes at both times of the year. This may indicate that the presence of the ram can influence luteal function during the postpartum period in ewes. Several studies have indicated that the ram effect is most effective when the ewes are anestrous (Wheaton et al., 1992; Scott and Johnstone, 1994; Lindsay, 1996), and the postpartum period seems to be the only time, besides gestation, when hair sheep ewes on St. Croix are anestrous.

Implications

The ram effect can be used as a management tool to induce estrous cyclicity in anestrous ewes in temperate areas of the world, but it seems that it is not as effective in ewes located at lower latitudes. The ewes do not express a seasonal pattern to their estrous cycles, which is probably the reason they do not respond as well to the ram effect. There was some

response to the presence of the ram in the ewes, as evidenced by higher concentration of progesterone postpartum. The fact that the progesterone response was influenced by time of year indicates that hair sheep ewes in the tropics may be responsive to the slight change in photoperiod of the region, but this resulted in a minimal response to the ram effect during these times of the year.

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