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Superovulation in Sheep: Number and Weight of the Corpora Lutea and Serum Progesterone[1,2]

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Summary

To determine similarities and differences between non-superovulated and superovulated ewe models, data collected from several experiments (1989 through 2005) were analyzed. Mature non-pregnant non-superovulated (n = 91) or superovulated (n = 299) Western range-type ewes were used for evaluation of luteal function. To induce superovulation, ewes were injected twice daily with FSH on days 13 to 15 of the estrous cycle. At corpora lutea (CL) collection on day 5 or 10 of the estrous cycle, the number of CL was determined. For selected ewes, the CL were weighed and blood samples were collected for determination of progesterone (P4) concentration in serum. Each year, a similar ($P > 0.1$) number of ovulations/ewe was induced by FSH treatment (range from 12.4 ± 2.0 to 20 ± 2.5/year). Superovulated ewes had greater ($P < 0.001$) number of CL than non-superovulated ewes (16.2 ± 0.5 vs. 1.9 ± 0.1). Weight of CL on day 5 of the estrous cycle was similar for superovulated and non-superovulated ewes (252.2 ± 4.1 vs. 224.7 ± 15.6 mg/CL), but on day 10, weight of CL from superovulated ewes was less ($P < 0.05$) than from non-superovulated ewes (379.9 ± 4.0 vs. 598.7 ± 18.5 mg/CL). Luteal tissue mass per ewe was greater ($P < 0.001$) for superovulated than non-superovulated ewes on days 5 (3.7 ± 0.4 vs. 0.5 ± 0.1 g) and 10 (6.1 ± 0.5 vs. 1.2 ± 0.1 g) of the estrous cycle. Serum P4 concentration on day 5 of the estrous cycle did not differ statistically ($P > 0.1$) for superovulated vs. non-superovulated ewes (2.3 ± 1.1 vs. 1.3 ± 0.1 ng/ml), but on day 10 tended to be greater ($P < 0.06$) in superovulated than non-superovulated ewes (5.8 ± 1.3 vs. 3.8 ± 0.3 ng/ml). When P4 concentration in serum was expressed per g of luteal tissue mass, values were similar for non-superovulated and superovulated ewes on days 5 and 10 of the estrous cycle. Moreover, all P4 values were greater ($P < 0.05$) on day 10 than on day 5 of the estrous cycle. Thus, despite some differences in CL number and CL weight, the major function of the CL, P4 production does not seem to be altered in superovulated ewes compared with non-superovulated ewes. Therefore, these data indicate that our superovulated ewe model may be used for studies of luteal function.

Keywords: Superovulation, Corpora Lutea, Serum Progesterone, Ewe

Introduction

Assisted reproduction technologies (ART) have been used in agriculture for many decades to increase reproductive potential of domestic farm animals (Gordon, 1997, 2005; Grazul-Bilska, 2004). In sheep, use of these techniques can help enhance reproductive efficiency (Cognie et al., 2003). Superovulation protocols allow one to take advantage of the relatively short gestation length of sheep and utilize the ewe to her fullest potential (Gordon, 1997; Gonzales-Bulnes et al., 2004).

Superovulation was developed approximately 55 years ago and has been implemented in sheep research and production (Driancourt and Fry, 1992; Gordon, 1997, 2005). Treatment with FSH or pregnant mare serum gonadotropin (PMSG) causes multiple follicles to develop followed by ovulation and creation of multiple corpora lutea (CL). Thus, the superovulated ewe had 5 to 27 CL (Stormshak et al., 1963; Hild-Petito et al., 1987; Jablonka-Shariff et al., 1993; Gonzales-Bulnes et al., 2004), and peripheral progesterone (P4) concentration was greater in superovulated than non-superovulated ewes (Stormshak et al., 1963; McClellan et al., 1975). However, morphology of CL was similar for superovulated and non-superovulated ewes (McClellan et al., 1975; Hild-Petito et al., 1987). Furthermore, superovulation did not affect circulating P4 concentration when expressed per mg of luteal tissue, basal P4 secretion by small and large luteal cells, and P4 concentration in luteal tissues (Stormshak et al., 1963; Hild-Petito et al., 1987). Hild-Petito et al. (1987) also demonstrated that small luteal cells differ in size and responsiveness to LH in superovulated compared to non-superovulated ewes. Our study was designed to further determine similarities and differences of CL development and function in superovulated vs. nonsuperovulated ewe models, and to provide additional information about these two models.

The aims of this study were to determine the number and weight of CL and serum P4 concentration in superovulated vs. non-superovulated ewes across several years.

Materials and Methods

Animal Treatment and Tissue Collection

The Institutional Animal Care and Use Committee at NDSU approved all animal procedures in this study. From 1988 to 2005 mature, non-pregnant, Western range-type ewes (n = 390) of mixed breeds (predominantly Targhee x Rambouillet) were used for several experiments during breeding season (September through January) to evaluate luteal function. A portion of ewes was non-superovulated (n = 91), and a portion of ewes was superovulated (n = 299). To induce superovulation, ewes were injected twice daily (morning and evening) with FSH-P (FSH with 10 percent luteinizing hormone) purchased from Schering (Kenilworth, NJ; 1989 through 1994; n = 128 ewes) or Sioux Biochemical (Sioux Center, IA; 1996 through 2005; n = 171 ewes) on days 13 (5 units/injection, day 0 = estrus), 14 (4 units/injection) and 15 (3 units/injection) of the estrous cycle (total dose = 24 units) to induce superovulation (Grazul-Bilska et al., 1991, 2001). Standing estrus (day 0 of the estrous cycle) was determined by using vasectomized rams. Ewes were fed a ration of mixed forage and cracked corn which was designed to meet the nutritional requirement for non-pregnant ewes (NRC, 1985), and had free access to a salt-mineral mixture and to water.

At CL collection on days 5 or 10 of the estrous cycle, each CL was dissected from ovarian tissues separately, and number of CL was determined for all non-superovulated (n = 91) and superovulated (n = 299) ewes. Individual CL were weighed for a portion of non-superovulated (n = 86) and superovulated (n = 87) ewes. Blood samples were collected for selected non-superovulated (n = 24) and superovulated (n = 15) ewes on days 5 and 10 of the estrous cycle to determine serum P4 concentration.

Progesterone RIA

Progesterone concentrations in extracted serum were measured as previously reported (Jablonka-Shariff et al., 1993; Vonnahme et al., 2006). Sensitivity of the assay was 12.5 pg/tube. The intra- and inter-assay coefficients of variation ranged from 3.4 percent to 6.4 percent, and from 7.1 percent to 12.6 percent, respectively.

Statistical Analysis

Data were analyzed using the general linear model (GLM) procedure of SAS (SAS, 2006), and presented as means ± SEM throughout the manuscript. When the F-test was significant ($P < 0.05$), differences between specific means were evaluated by using least significant differences test (Kirk, 1982).

Results

From 1989 to 2005, similar ($P > 0.1$) number of ovulations/ewe, measured by the CL number, was induced using both FSH preparations (Figure 1). Source of FSH did not affect number and weight of CL. A similar number of ovulations ($P > 0.1$) was

Figure 1. Mean number of FSH-induced ovulations/ewe, measured by the number of CL, from 1989 to 2005 (number in each bar indicates the number of superovulated ewes in a specific year).

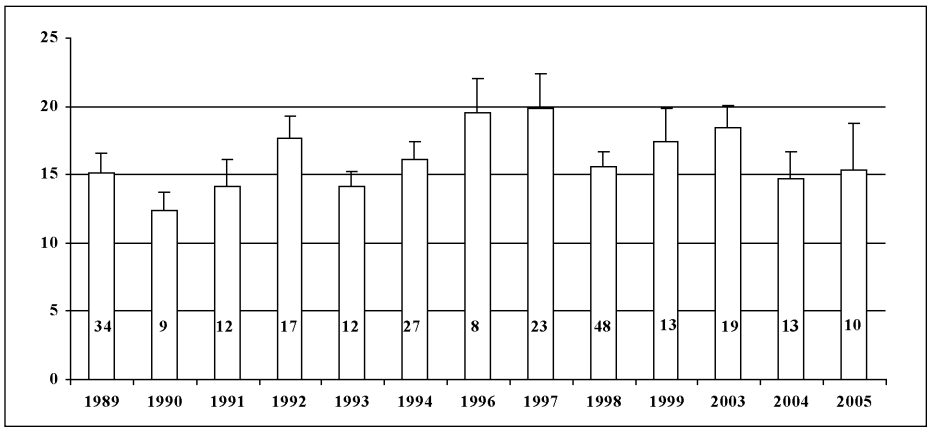


Table 1. The effects of superovulation on number and weight of the CL on days 5 and 10 of the estrous cycle.

	Non-superovulated	Superovulated
Number of CL	1.9 ± 0.1[a] (n = 91 ewes)	16.2 ± 0.5[b] (n = 245 ewes)
Weight of individual CL (mg)		
Day 5	224.7 ± 15.6 (n = 39 CL)	252.2 ± 4.1 (n = 443 CL)
Day 10*	598.7 ± 18.5[c] (n = 123 CL)	379.9 ± 4.0[d] (n = 936 CL)
Luteal tissue mass/ewe (g)**		
Day 5	0.46 ± 0.06[a] (n = 24 ewes)	3.74 ± 0.37[b] (n = 29 ewes)
Day 10*	1.20 ± 0.05[a] (n = 62 ewes)	6.12 ± 0.49[b] (n = 58 ewes)

[a,b] $P < 0.001$; [c,d] $P < 0.05$; means ± SEM with different superscripts differ within a row.
* $P < 0.05$; means ± SEM for CL weight and luteal tissue mass on day 10 are greater than on day 5 of the estrous cycle within a column.
** Luteal tissue mass is a sum of weight of all CL from individual ewe.

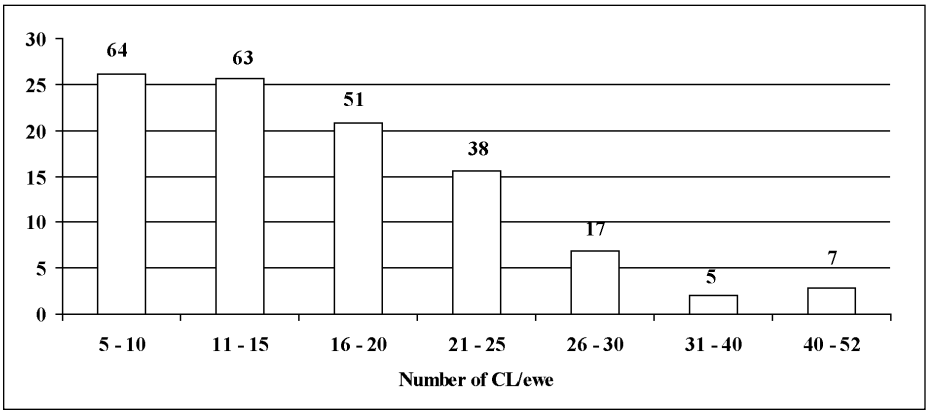
achieved when sheep were treated with FSH-P from Schering or Sioux Biochemical (15.3 ± 0.7 and 16.9 ± 0.8 CL per ewe, respectively). Therefore, data for these two FSH preparations were combined for further analysis.

The number of CL and the weight of each CL for non-superovulated and superovulated ewes on days 5 and 10 of the estrous cycle are presented in Table 1. The number of CL per ewe was greater ($P < 0.001$) in superovulated ewes than non-superovulated ewes (Table 1). The percentage of non-superovulated ewes with 1, 2, 3 or 4 CL was 30 percent (n = 27), 56 percent (n = 51), 9 percent (n = 8), and 5.5 percent (n = 5), respectively. The number of CL for superovulated ewes which responded to FSH-treatment (n = 245), ranged from 5 to 52/ewe; 88 percent of ewes had 5 to 25 CL, and 12 percent had 26 to 52 CL (Figure 2). On day 5 of the estrous cycle, the weight of the individual CL was similar for non-superovulated and superovulated ewes. But on day 10, individual CL weight from the non-superovulated ewes was greater ($P < 0.05$) than from the superovulated ewes (Table 1). Total luteal tissue weight per ewe was greater ($P < 0.001$) for superovulated than non-superovulated ewes on days 5 and 10 of the estrous cycle (Table 1). The individual CL weight and total luteal tissue weight per ewe were greater on day 10 than on day 5 of the estrous cycle for both non-superovulated and superovulated ewes (Table 1).

Serum P4 concentrations, and P4 secretion expressed per g of luteal tissue mass in non-superovulated and superovulated ewes is presented in Table 2. Serum P4 concentrations were similar for non-superovulated and superovulated ewes on day 5 of the estrous cycle. However, on day 10 of the estrous cycle, serum P4 concentration tended to be greater ($P < 0.06$) in superovulated than non-superovulated ewes (Table 2). When P4 secretion was expressed per g of total luteal tissue mass per ewe, P4 values were similar for non-superovulated and superovulated ewes on days 5 and 10 of the estrous cycle. In addition, all P4 values were greater on day 10 than on day 5 of the estrous cycle for both non-superovulated and superovulated ewes.

A proportion of ewes (overall 18.1 percent; n = 54) did not respond to FSH induction of superovulation, which was manifested by the presence of one to four CL after FSH treatment. During years 1988 to 1994 (when FSH-P from Schering was used) and during years 1995 to 2005 (when FSH-P from Sioux Biochemical was used) lack of superovulatory response to FSH treatment was similar ($P > 0.1$) between these two FSH preparations (13.2 percent ± 2.8 percent and 19.5 percent ± 3.1 percent, respectively).

Figure 2. Percentage of superovulated ewes (n = 245) with multiple CL (number above bar indicates number of sheep).



Discussion

Our study demonstrated no differences in number of CL in response to superovulation treatment with FSH from two different sources/preparations. Furthermore, the response to superovulatory treatment was consistent throughout 17 years of FSH application in our research program. Thus, these FSH preparations were equally active for induction of superovulation. The purified FSH preparation is optimal when it includes less than 10 percent LH (Donaldson, 1991; Boscos et al., 2002) because the ratio of FSH:LH is critical for the development of preovulatory follicle and ovulation (Donaldson, 1991; Senger, 2003; D'Alessandro et al., 2005). Therefore, in this study we used the FSH preparations containing less than 10 percent LH.

In this study, the number of CL ranged from one to four (average 1.9) and from to 5 to 52 (average 16.2) for non-superovulated and superovulated ewes, respectively. Similar average numbers of CL per superovulated ewe were

Table 2. The effects of superovulation on progesterone (P4) concentration in serum (ng/ml) on days 5 (n = 12 for non-superovulated and n = 5 for superovulated) and 10 (n = 12 for non-superovulated and n = 10 for superovulated) of the estrous cycle.

		Non-superovulated	Superovulated
P4 (ng/ml)			
	Day 5	1.28 ± 0.13	2.32 ± 1.06
	Day10**	3.82 ± 0.33 [a]	5.75 ± 1.26 [b]
P4 (per g of luteal tissue)*	Day 5	0.22 ± 0.02	0.27 ± 0.05
	Day 10**	0.68 ± 0.04	0.87 ± 0.26

* Calculated by dividing P4 concentration in serum by luteal tissue mass per ewe.
** $P < 0.001$; means ± SEM for P4 values on day 10 are greater than on day 5 of the estrous cycle within a column.
[a,b] $P < 0.06$; means ± SEM with different superscripts differ within a row.

reported in other studies using a multiple FSH-treatment protocol (Stormshak at al., 1963; Amiridis et al., 2002; Ammoun et al., 2006; Mossa et al., 2006; Veiga-Lopez et al., 2006). However, when lower doses of FSH (e.g., 2.5 to 10 units), or one injection of FSH or PMSG were used, number of CL varied from one to six (Boscos et al., 2002; Riesenberg et al., 2001; Hild-Petito et al., 1987). Thus, number of CL after induced superovulation depends on dose and type of hormone used (e.g., FSH or PMSG); frequency of treatment; and also time of treatment related to the stage of the estrous cycle. Furthermore several factors, including variations in follicular (e.g., number of follicular waves or follicle sizes) and hormonal (e.g., level of peripheral pituitary and ovarian hormones) dynamics in individual ewes, may contribute to variability in the CL number/ewe after FSH treatment (Driancourt, 2001; Riesenberg et al., 2001; Amiridis et al., 2002; Cognie et al., 2003; Gonzales-Bulnes et al., 2004). However, this subject requires additional study.

In the present study, the weight of individual CL and serum P4 concentration in superovulated ewes were similar to non-superovulated ewes on day 5 of the estrous cycle. However, on day 10 of the estrous cycle, CL weight was less in superovulated than non-superovulated ewes, but serum P4 concentration was greater in superovulated than non-superovulated ewes. Similar CL weight for non-superovulated and superovulated ewes on day 10 of the estrous cycle, but greater serum P4 concentrations in superovulated than non-superovulated ewes has been demonstrated by Hild-Petito et al. (1987). In contrast, Stormshak et al. (1963) reported lower CL weight of individual CL in superovulated compared to non-superovulated ewes throughout the estrous cycle. These discrepancies in CL weight and P4 concentration are likely due to the different superovulation protocol used in these studies. In fact, superovulation was induced using PMSG followed by hCG treatment by Hild-Petito et al. (1987) but using multiple injections of ovine pituitary extract followed by hCG treatment by Stormshak et al. (1963). Thus, superovulation protocols may affect not only the number of CL, but also weight of CL.

On day 5 of the estrous cycle, the CL is still rapidly growing and differentiating (Jablonka-Shariff et al., 1993), therefore, differences in CL weight or serum P4 concentrations could not be observed during the early luteal phase for non-superovulated and superovulated ewe models. By day 10, when the CL reaches its fully functional and differentiated stage, differences in weight and serum P4 concentrations for non-superovulated and superovulated ewes were observed. Thus, as compared to non-superovulated ewes, when multiple CL (i.e., more than four) are developing in superovulated ewes, growth seems to be limited, and they fail to achieve their typical weight. Furthermore, it seems that because there are more CL on the superovulated ovary that they had less room to grow and consequently grew smaller to approximately 0.6x of the size of individual CL found on non-superovulated ovaries. Additionally, reduced luteal weights on day 10 of the estrous cycle in superovulated ewes is likely associated with control of luteal function by LH (Niswender and Nett, 1994). Since greater luteal tissue mass can produce more P4, as observed in our and other studies (Amiridis et al., 2002) in superovulated ewes, P4 through negative feedback may inhibit LH secretion, which in turn may limit growth of the CL. However, this concept requires further investigation.

When P4 secretion was expressed per g of luteal tissue mass, P4 values were similar for superovulated and non-superovulated ewes on days 5 and 10 of the estrous cycle. Similar observations were reported by Hild-Petito et al. (1987) for the CL from day 10 of the estrous cycle. Furthermore, Stormshak et al. (1963) demonstrated that luteal P4 concentration was similar for superovulated and non-superovulated ewes. Thus, total luteal tissues in superovulated ewes secrete amounts of P4 similar to non-superovulated ewes, which is likely due to tight control by LH. Furthermore, it has been demonstrated that CL structure and luteal function measured by P4 secretion and *in vitro* unresponsiveness of large luteal cells to LH and dbcAMP treatment were similar for non-superovulated and superovulated ewes (McClellan et al., 1975; Hild-Petito et al., 1987). We have also demonstrated in several studies, that luteal cells from superovulated ewes responded to LH or dbcAMP stimulation by increasing P4 secretion in vitro (Grazul-Bilska et al., 1991, 1995, 1996). However, the mean cell diameter and LH stimulation of P4 secretion by small luteal cells differed between superovulated and non-superovulated ewes (Hild-Petito et al., 1987). Therefore, these data indicate that despite some differences, function of CL reflected by P4 secretion in superovulated ewes is similar to function of CL in non-superovulated ewes.

Several other studies demonstrated that peripheral P4 concentrations were enhanced in superovulated ewes during several stages of the estrous cycle (Stormshak et al., 1963; McClellan et al., 1975; Hild-Petito et al., 1987; Amiridis et al., 2002). Furthermore, Amiridis et al.

(2002) showed a positive relationship between number of CL and serum P4 levels on day 5 of the estrous cycle. In our study, positive correlations were also observed between P4 secretion and CL number and luteal tissue mass for combined data for days 5 and 10 of the estrous cycle. This clearly demonstrates that the amount of P4 circulating in the blood is relative to the total luteal tissue mass. However, as discussed above, secretion of P4 is likely limited by LH and possibly other factors in superovulated ewes.

In this study, approximately 18 percent of ewes did not respond to the FSH treatment as indicated by presence of only one to four CL. In agreement with our data, Cognie (1999) reported that about 20 percent of ewes did not respond to superovulatory treatment. It has been hypothesized that a lack of superovulatory response to FSH by some ewes is due to a heterogeneity in the morphological features of the ovulatory follicles or to the number of small antral follicles present in the ovaries when FSH treatment was initiated (Draincourt, 2001; Cognie et al., 2003). Also season, breed, and nutritional treatments may all contribute to the variability of responsiveness to FSH treatments (Cognie, 1999). Future studies should be undertaken to determine why a relatively large proportion of ewes does not respond to the FSH-treatment.

Summary

In summary, this study demonstrated that 1) a consistent number of ovulations measured by the number of CL was induced across 17 years of using the FSH treatment; 2) the number of CL was greater in superovulated than non-superovulated ewes; 3) weight of individual CL was similar on day 5 but less on day 10 in superovulated than non-superovulated ewes; 4) serum P4 concentration was similar on day 5 but greater on day 10 in superovulated than non-superovulated ewes; 5) P4 secretion expressed per g of luteal tissue mass was similar on days 5 and 10 for superovulated and non-superovulated ewes, and 6) 18 percent of ewes did not respond to FSH treatment in our superovulation protocol. Thus, variation in number of CL and weights of luteal tissue between non-superovulated and superovulated ewes did not significantly affect P4 secretion when expressed per g of luteal tissue mass. Therefore, this superovulated ewe model is a reasonable model for the study of luteal function and is also helpful for generating larger amounts of luteal tissue per animal for use in complex studies of the CL function.

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