Incidence of Premature Luteal Regression in Ewes Superovulated with a Single Injection of Follicle-Stimulating Hormone Combined with Equine Chorionic Gonadotropin

Yutaka FUKUI, Midori OKADA and Naohisa ISHIDA

Laboratory of Animal Genetics and Reproduction, Obihiro University of Agriculture and Veterinary Medicine, Obihiro 080, Japan

Abstract. The incidence of premature corpora lutea (CL) regression was investigated in three different breeds (Merino x polled Dorset: MD, Suffolk: S, and South-Down: SD), parous and non-parous ewes (a total of 46 ewes) treated for superovulation during and out of the breeding season. Superovulation was induced with a single injection of 20 mg porcine follicle-stimulating hormone (pFSH) 2 days and 500 IU equine chorionic gonadotropin (eCG) 1 day, respectively before the removal of synthetic progestogen (40 mg FGA) vaginal sponges inserted for 12 days. All ewes were inseminated into the uterus with fresh-diluted or frozen-thawed semen by laparoscope, and a number of newly formed and regressed CL were recorded. Breed difference significantly ($P < 0.005$) affected the incidence of premature regressed CL; S (6.3%, $n=16$) ewes were less in the proportion of the incidence of regressed CL than MD (37.5%, $n=16$; $P < 0.05$) and SD (71.4%, $N=14$; $P < 0.01$) ewes. The use of non-parous ewes and treatment during autumn tended to result in higher rates of incidence of premature CL regression than the use of parous ewes and treatment during spring. These results indicate that the incidence of premature CL regression in superovulated ewes was influenced by not only season and parity but also by breed of ewes used for superovulation treatment.

Key words: Superovulation, Corpus Luteum, Season, Breed, Sheep.

(J. Reprod. Dev. 44: 407–412, 1998)

Multiple injections of follicle-stimulating hormone (FSH) or a single injection of equine chorionic gonadotropin (eCG) has been conventionally used for superovulation in ewes [1–4]. FSH has been administered by decreasing or constant doses in 6–8 injections with approximately 12 h interval due to the short half-life [1, 5]. However, the multiple injection of FSH is a labour-consuming scheme for induction of superovulation. On the other hand, a single injection of a high dose (1,000 to 1,500 IU) eCG can also induce superovu-

lation, but its relatively high level of estradiol-17 β (E_2) remaining in the unovulatory follicles and in the blood has impaired oocyte maturation, time of ovulation and the yield of fertilized ova [6, 7].

Ryan *et al.* [8] and Maxwell *et al.* [9] reported that a single injection of 12–18 mg FSH in combination with 300–800 IU eCG resulted in a satisfactory superovulation and embryo recovery as compared with multiple injections of FSH combined with eCG in ewes. Similar results have been reported in ewes [10] and goats [11]. However, prematurely regressed CL has been observed in superovulated ewes [12–14], cows [15] and goats [16–18]. Ryan *et al.* [13] reported that the proportion of ewes that ovulated and subsequently had

premature regressed CL was significantly ($P < 0.01$) greater in autumn than in spring. Although the detailed mechanism of the premature CL regression in superovulated ewes is not fully understood, a role of an uterine luteolysin, a delay of luteinizing hormone (LH) and abnormal steroid levels (especially E_2) during the preovulatory period with the increased doses of gonadotropin have been considered to be involved for the incidence of premature CL regression.

The endocrinological aspects by the simple superovulation method with FSH and eCG combined treatment has not yet been fully investigated. Recently, we administered a single injection of 20 mg porcine FSH (pFSH) 2 days and 500 IU eCG 1 day, respectively before removal of progestogen vaginal sponges in Suffolk ewes during the breeding season, and found that the proportion of normal recovered and fertilized embryos (63%) was significantly ($P < 0.05$) higher than the simultaneous injections of pFSH and eCG 2 days (41%) before sponge removal [19, 20]. To determine whether or not some factors (season, parity and breed of ewes) affect the incidence of premature CL regression in ewes treated with single injections of pFSH and eCG on two days and one day, respectively before the termination of progestogen treatment would be worthwhile for embryo recovery in the superovulation treatment.

The objective of the present study was to investigate the incidence of premature CL regression in three different breeds of parous and non-parous ewes superovulated with our established simple method [19, 20] during (autumn) and out of (spring) the breeding season.

Materials and Methods

Animals

Sixteen Merino x polled Dorset (MD: 9 parous and 7 non-parous), 16 Sufflok (S: 9 parous and 7 non-parous) and 14 South-Down (SD: 10 parous and 4 non-parous) ewes were used in this study (Total $N=46$). Twenty-one ewes (5 MD, 9 S and 7 SD) and 25 (11 MD, 7 S and 7 SD) ewes were treated for superovulation during anestrus (May to July) and estrus (September to December) seasons in 1997, respectively. In each season, ewes were brought into the Laboratory Farm one month before commencing the study and kept under the

same conditions. They were fed 1 kg hay per head (twice daily) and a concentration pellet containing 15% crude protein (about 200 g per head per day), and allowed free access to water, salt and mineral blocks.

Treatment

In both seasons, the ewes were treated with vaginal sponges impregnated with 40 mg fluorogesterone acetate (FGA: "Chronogest": Intervet International B.V., The Netherlands) for 12 days. At 2 days (Day -2) and 1 day (Day -1) before sponge removal (Day 0: the day of sponge removal), a single injection of pFSH (Antorin, Denka Chemical Co., Japan) and 500 IU eCG (Serotropin: Teikoku-zoki Co., Japan) were administered intramuscularly. The ewes were then examined for estrus incidence from -12 h to 36 h (0 h: the time of sponge removal) using 2–3 entire rams aproned and equipped with marking harnesses and crayons. At 24 h after sponge removal, all ewes were given an injection of 100 μ g gonadotrophin releasing hormone (GnRH: Conceral, Takeda Industrial Co., Japan). At 36 h after sponge removal, artificial insemination (AI) with fresh-diluted or frozen-thawed semen was performed using a laparoscope by the method of Maxwell *et al.* [21].

Laparotomy

On Day 8 after sponge removal (6.5 days after AI), mid-ventral laparotomy was carried out under a local anaesthesia with 2% xylocaine. Numbers of newly formed and regressed CL were recorded for all ewes. CL which were small and pale in morphological appearance, were considered as prematurely regressed CL [12].

Statistical analysis

The total proportions of ewes classified as having regressed CL were compared between parous and non-parous ewes, between autumn and spring and among the three breeds of ewes by Fisher's exact probability test. Mean ovulation rates in ewes with normal CL were also compared among the breeds of ewes and between seasons using a Student's *t*-test.

Results

One out of 4 non-parous SD ewes treated dur-

Table 1. Proportions of the incidence of premature regressed CL in superovulated parous and non-parous ewes (combined data of spring and autumn)

Ewes	Breeds* ¹			Total (%)
	MD	S	SD	
Parous	2/9* ²	0/9	6/10	8/28 (28.6)
Non-parous	4/7	1/7	4/4	9/18 (50.0)
Total (%)	6/16 (37.5) ^a	1/16 (6.3) ^b	10/14 (71.4) ^c	17/46 (37.0)

*1: The breeds of ewes: MD; Merino x polled Dorset, S; Suffolk, SD; South-Down.

*2: Number of ewes with premature regressed CL/number of ewes treated.

a vs b: P<0.05, b vs c: P<0.01, a vs c: not significant.

Table 2. Proportions of the incidence of premature regressed CL in superovulated ewes during spring and autumn (combined data of parous and non-parous ewes)

Season	Breeds* ¹			Total (%)
	MD	S	SD	
Spring	1/5* ²	0/9	4/7	5/21 (23.8)
Autumn	5/11	1/7	6/7	12/25 (48.0)
Total (%)	6/16 (37.5) ^a	1/16 (6.3) ^b	10/14 (71.4) ^c	17/46 (37.0)

*1: The breeds of ewes are the same as in Table 1.

*2: Number of ewes with premature regressed CL/number of ewes treated.

a vs b: P<0.05, b vs c: P<0.01, a vs c: not significant.

ing autumn did not show estrus during the observation periods up to 36 h after sponge removal. The other all ewes showed estrus between 12 and 30 h after sponge removal.

The incidence of premature CL regression in parous and non-parous ewes in three different breeds is shown in Table 1. Non-parous ewes had a higher proportion of ewes with regressed CL than that in parous ewes (50.0 vs. 28.6%), but they were not significantly different. On the other hand, breed difference resulted in significant differences in the proportions of ewes with regressed CL between MD and S (P<0.05), and between S and SD (P<0.01). There was no significant difference between MD and SD. Season tended to affect the incidence of premature regressed CL (spring: 23.8% vs. autumn: 48.0%; P<0.10) as shown in Table 2.

Table 3 shows the mean ovulation rate in the three different breeds of ewes superovulated with

Table 3. Mean ovulation rate ($\pm$ SEM) of three different breeds of ewes superovulated with normal corpora lutea during spring and autumn (combined data of parous and non-parous ewes)

Ewes	Season (no. of ewes ovulated)		Total
	Spring	Autumn	
MD*	19.3 $\pm$ 4.3 (4)	12.3 $\pm$ 4.4 (6)	15.1 $\pm$ 5.4 (10) ^A
S*	11.0 $\pm$ 5.5 (9) ^a	20.8 $\pm$ 2.7 (6) ^b	14.9 $\pm$ 6.7 (15) ^A
SD*	6.3 $\pm$ 3.5 (3)	9 (1)	7.0 $\pm$ 3.2 (4) ^B
Total	12.2 $\pm$ 6.5 (16)	16.0 $\pm$ 5.8 (13)	

*MD: Merino x polled Dorset, S: Suffolk, SD: South-Down
a vs b: P<0.001, A vs B: P<0.01.

normal CL during spring and autumn. SD ewes had significantly (P<0.01) lower mean ovulation rate (7.0 $\pm$ 3.2) as compared with MD (15.1 $\pm$ 5.4) and S (14.9 $\pm$ 6.7) ewes. Season did not affect the ovulation rates (spring: 12.2 $\pm$ 6.5 and autumn: 16.0 $\pm$ 5.8), but S ewes had a greater (P<0.001) ovulation rate in autumn (20.8 $\pm$ 2.7) than in spring (11.0 $\pm$ 5.5).

Discussion

Premature luteal regression has been reported in superovulated ewes [12–14, 22], and has impaired the yield of normal fertilized embryos. Jabbour *et al.* [12] and Ryan *et al.* [13] reported that more ewes had regressed CL in autumn (24% and 37%, respectively) than in spring (6% and 21%, respectively). In their reports, 12 or 18 mg pFSH was administered with multiple injections. A single injection of pFSH and eCG has been successfully attempted to yield similar ovulation rates compared with a combined treatment by multiple injections of pFSH and a moderate dose (200–800 IU) of eCG [8–10]. In the present study using three different breeds of ewes, 20 mg pFSH combined with 500 IU eCG was administered as a single injection, respectively 2 days and 1 day before the removal of progestogen sponges. The present results show a similar phenomenon that the proportion of ewes with premature regressed CL tended to be higher (P<0.10) in autumn than in spring. Armstrong *et al.* [23] suggested that the incidence of ewes with regressed CL might be related to excessive levels of estrogen bringing about luteolysis. Watanabe *et al.* [19] observed a biphasic increase of E₂ secretion in superovulated ewes when pFSH and eCG were

given on Day -2 and Day -1, respectively before sponge removal. The higher estrogen levels from persistent large follicles have been considered to enhance ova transport through the oviducts and become detrimental to fertilization and the development of embryos [7, 24, 25]. It has been reported that an administration of exogenous estrogen shortly after ovulation induced premature luteal regression in cows [26] and in rhesus monkeys [27]. Battye *et al.* [28] considered that premature luteal regression occurred by the premature release of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) in the uterus under the influence of higher levels of estrogen. The criteria to identify the regressed CL used in the present study were their morphological appearances (small and pale) according to the report of Jabbour *et al.* [12]. Although the criteria attempted was distinguishable between the regressed and newly formed CL, P_4 and E_2 profiles following superovulation should be clarified in the future study in order to firmly support the hypothesis that excessive levels of E_2 is related to the incidence of the premature CL regression and to identify if and when the regression occurs.

The present study showed a new finding that breeds of ewes for superovulation treatment also significantly affected the incidence of premature CL regression; 71.4% of SD ewes had the highest rate of prematurely regressed CL, whereas S ewes had only 6.3%. The combined treatment with a single pFSH (20 mg) and eCG (500 IU) injections might be over-dosed in SD ewes. Ryan *et al.* [13] obtained the maximum rate of recovery, fertilized embryos with a moderate dose of eCG (800 IU) in combination with 12 or 18 mg pFSH in Merino ewes, but reported that the incidence of regressed CL increased as a total dose of gonadotropins increased. The cause of the premature CL regression has not been defined. However, a nutritional component to this phenomenon has been suggested. Ryan *et al.* [13] reported that mean plasma glucose levels were lower in ewes with regressed CL than in those with normal CL, probably due to the fact that pasture quality is lower in autumn

than in spring. Thus, supplementary feeding such as lupin grains during autumn has been recommended to reduce the incidence of luteal regression in superovulated ewes [12, 29]. Although a clear cause for the significant difference in the incidence of regressed CL among the three breeds of ewes used in the present study is not clarified, nutritional status of the treated ewes might have influenced on the proportions of ovulation and the incidence of premature CL regression. Breeds of ewes have been one of the factors influencing the ovarian response and ovulation rate following superovulation treatment. It is known that sheep of high fecundity such as Merino ewes carrying the F gene are more sensitive to exogenous gonadotropins than animals of low fecundity [30–32]. However, the present study is the first report indicating that the incidence of premature CL regression in superovulated ewes is also influenced by breed effect.

In the present study, non-parous ewes had more premature regressed CL as compared with parous ewes (50.0% and 28.6% respectively), although the difference was not significant. These results may indicate that body weight and body condition relating to nutritional status also influence the incidence of premature luteal regression. For non-parous ewes, the present doses of pFSH and eCG may be over-dosed, so that either pFSH or eCG dose should have been reduced.

In conclusion, the present results indicate that not only season and parity but also breed of ewes affect the incidence of premature luteal regression in ewes superovulated with a single pFSH injection combined with eCG.

Acknowledgment

The present work was supported by a grant from the Hokkaido Foundation for the Promotion of Scientific and Industrial Technology (Hokscitec), Japan.

References

1. Torrie S, Cognie Y, Colas G. Transfer of superovulated sheep embryos obtained with different FSH-P. *Theriogenology* 1987; 27: 407–419.
2. Walker SK, Smith DH, Frensham A, Ashman RJ, Seamark RF. The use of synthetic gonadotrophin releasing hormone treatment in the collection of

- sheep embryos. *Theriogenology* 1989; 31: 741–752.
3. **Jabbour HN, Evans G.** Ovarian and endocrine responses of Merino ewes to treatment with PMSG and/or FSH-P. *Anim Reprod Sci* 1991; 2: 93–106.
 4. **Fukui Y, Tashiro Y, Kimura H, Miyamoto A.** Effects of progestogen treatment and season on superovulatory responses of ewes and developmental capacity of early embryos recovered. *J Reprod Dev* 1994; 40: 251–257.
 5. **Boland MP, Roche JF.** Embryo production in vivo: problems and prospects. In: A Lauria and F. Gandolfi (eds.), *Embryonic Development and Manipulation in Animal Production. Trends in Research and Application*. London: Portland Press; 1992: 195–206.
 6. **Shelton JN, Moore NW.** The response of the ewes to pregnant mare serum and to horse anterior pituitary extract. *J Reprod Fertil* 1967; 14: 175–177.
 7. **Moor RM, Osbourne JC, Crosby IM.** Gonadotrophin-induced abnormalities in sheep oocytes after superovulation. *J Reprod Fertil* 1985; 74: 167–172.
 8. **Ryan JP, Bilton RJ, Hunton JR.** Superovulation of ewes with a combination of PMSG and FSH-P. In: Lindsay DR, Pearce DT (eds.), *Reproduction in Sheep*, Cambridge University Press; 1984: 338–341.
 9. **Maxwell WMC, Szell A, Hunton JR, Ryan JP.** Artificial breeding: embryo transfer and cloning. In: Oldham CM, Martin GB, Purvis IW (eds.), *Reproductive Physiology of Merino Sheep*. Perth: The University of Western Australia; 1990: 217–237.
 10. **Yamada A, Kawana M, Tamura Y, Miyamoto A, Fukui Y.** Effect of single or multiple injection of follicle stimulating hormone combined with pregnant mare serum gonadotropin on superovulatory response, and normal and freezable embryos in ewes. *J Reprod Dev* 1996; 42: 81–87.
 11. **Batt PA, Killeen ID, Vameron AWN.** Use of single or multiple injection of FSH in embryo collection programs in goat. *Reprod Fertil Dev* 1993; 5: 49–56.
 12. **Jabbour HN, Ryan JP, Evans G, Maxwell WMC.** Effects of season, GnRH administration and lupin supplementation on the ovarian and endocrine responses of Merino ewes treated with PMSG and FSH-P to induce superovulation. *Reprod Fertil Dev* 1991; 3: 699–707.
 13. **Ryan JP, Hunton JR, Maxwell WMC.** Increased production of sheep embryos following superovulation of Merino ewes with a combination of pregnant mare serum gonadotrophin and follicle stimulating hormone. *Reprod Fertil Dev* 1991; 3: 551–560.
 14. **Ledda S, Naitana S, Loi P, Dattena M, Gallus M, Brara A, Cappai P.** Embryo recovery from superovulated mouflons (*Ovis gmelini musimon*) and viability after transfer into domestic sheep. *Anim Reprod Sci* 1995; 39: 109–117.
 15. **Booth WP, Newcomb R, Strange H, Rowson LEA, Sacher HE.** Plasma oestrogen and progesterone in relation to superovulation and egg recovery in the cow. *Vet Rec* 1975; 97: 366–369.
 16. **Epplleston J.** Embryo transfer procedures in the goat: physiological and procedural differences in superovulation and transfer between sheep and goats. In: Shelton JN, Trounson AO, Moore NW, James JW (eds.), *Embryo Transfer in Cattle, Sheep and Goats*. Canberra: Australian Society of Reproductive Biology Publication; 1982: 41–43.
 17. **Armstrong DT, Pfizner AP, Warnes GM, Seamark RF.** Superovulation treatments and embryo transfer in Angora goats. *J Reprod Fertil* 1983; 67: 403–410.
 18. **Armstrong DT, Pfizner AP, Warnes GM, Ralph MM, Seamark RF.** Endocrine responses of goats after induction of superovulation with PMSG and FSH. *J Reprod Fertil* 1983; 67: 395–401.
 19. **Watanabe H, Miyamoto A, Ishida N, Okada M, Kimura H, Fukui Y.** A simple superovulation method of a single injection of follicle-stimulating hormone combined with equine chorionic gonadotropin for superovulation of Suffolk ewes during the breeding season: I. Effects of different treatments on the endocrine profiles. *J Reprod Dev* 1998; 44: 169–176.
 20. **Watanabe H, Ishida N, Okada M, Kimura H, Miyamoto A, Fukui Y.** A simple superovulation method of a single injection of follicle-stimulating hormone combined with equine chorionic gonadotropin for superovulation of Suffolk ewes during the breeding season: II. The ovarian responses and the embryo qualities. *J Reprod Dev* 1998; 44: 177–183.
 21. **Maxwell WMC, Butler LG, Wilson HR.** Intrauterine insemination of ewes with frozen semen. *J Agric Sci, Camb* 1984; 102: 233–235.
 22. **Trounson AO, Willardsen SM, Moor RM.** Effect of prostaglandin analogue cloprostenol on oestrus, ovulation and embryonic viability in sheep. *J Agric Sci, Camb* 1976; 86: 609–611.
 23. **Armstrong DT, Pfizner AP, Porter KJ, Janson PO, Seamark RF.** Ovarian responses of anoestrous goats to stimulation with pregnant mare serum gonadotrophin. *Anim Reprod Sci* 1982; 5: 15–23.
 24. **Evans G, Robinson TJ.** The control of fertility in sheep: endocrine and ovarian responses to progestagen-PMSG treatment in the breeding season and in anoestrus. *J Agric Sci, Camb* 1980; 94: 69–88.
 25. **Whyman D, Moore RW.** Effects of PMSG and the prostaglandin F-2 α analogue, cloprostenol, on superovulation, fertilization and egg transport in the ewe. *J Reprod Fertil* 1980; 60: 267–272.
 26. **Wiltbank JN.** Modification of ovarian activity in the bovine following injection of oestrogen and gonadotrophin. *J Reprod Fertil Suppl* 1966; 1: 1–8.

27. **Schoonmaker JN, Borgman KS, Steiner A, Karsh FJ.** Estradiol-induced luteal regression in the Rhesus monkey: evidence for an extra-ovarian site of action. *Endocrinology* 1982; 110: 708–715.
28. **Battye KM, Fairclough RJ, Cameron AWH, Trounson AO.** Evidence for prostaglandin involvement in early luteal regression of the superovulated nanny goat (*Capra hircus*). *J Reprod Fertil* 1988; 84: 425–430.
29. **Ryan JP, Maxwell WMC, Hunon JR.** Factors affecting the incidence of prematurely regressing corpora lutea in superovulated ewes. *Proc Aust Soc Reprod Biol* 1987; 19: 69 (abstract).
30. **Smith JF.** Selection for fertility and response to PMSG in Romney ewes. *Proc New Zealand Soc Anim Prod* 1976; 36: 247–251.
31. **Piper LR, Bindon BM, Cortis YM, Cheers MA, Nethery RD.** Response to PMSG in Merino and Booroola Merino crosses. *Proc Aust Soc Reprod Biol* 1982; 14: 82 (abstract).
32. **Kelly RW, Owens JL, Crosbie SF, McNatty KP, Judson N.** Influence of Booroola Merino genotype on the responsiveness of ewes to pregnant mares serum gonadotrophin, luteal tissue weight and peripheral progesterone concentration. *Anim Reprod Sci* 1983; 6: 199–207.