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Research Note

Repeated Injections of Pregnant Mare Serum Gonadotrophin (PMSG) Failed to Induce Antibody Production in Fall Lambing Ewes

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Pregnant Mare Serum Gonadotrophin (PMSG) is commonly used to induce estrus/puberty or superovulate beef cows (Gonzalez et al., 1994), dairy cows (Kummer et al., 1980; Saumande et al., 1984), gilts or sows (Holtz and Schlieper, 1991) or ewes (Evans and Robinson, 1980; Jabbour and Evans, 1990). Across species, variation in the success of recovering viable ova or embryos following PMSG can be attributed to hormonal profiles postinjection (Bever and Dieleman, 1987), age of treated animal (Lerner et al., 1986) or day of the cycle on which treatment is administered (Moor et al., 1984). Reduction in time of activity of PMSG has been attempted by administering PMSG antibodies, but success has been variable (Kummer et al., 1980; Jabbour and Evans, 1990; Kirkwood et al., 1994). Another factor that diminishes the effectiveness of inducing ovulation is the number of superovulatory treatments to which an animal has been subjected (Christie et al., 1979; Moor et al., 1984). The objective of this study was to examine whether conception rate of ewes induced to ovulate out of season in successive years could be attributed to presence of circulating antibodies to PMSG.

During three successive years, Suffolk and Rambouillet x Dorset ewes were implanted with Synchron-Mate-B[®] (SMB; CEVA Laboratories, Overland Park, KS) implants for 10 days and then injected with PMSG (Dr. Steve Webel, Animal Production Associates, Cropsey, IL) to induce estrus out of season (last two weeks of May). Due to the difference in body weights of the ewes, the white-faced ewes were injected with 500 IU of PMSG and the Suffolk ewes were injected with 750 IU of PMSG (average was 10.8 IU PMSG/kg body weight).

In the first year of the study, 27 of 55 ewes that received PMSG lambed in the fall. After weaning, blood samples were drawn and placed on ice and stored at 4° C for six hours. Serum was decanted after centrifugation at 1,500 x g for 30 minutes and stored at -20° C until assayed for presence of antibodies for PMSG.

In years 2 and 3, the majority of the ewes were again injected with PMSG. Sera were obtained at three times from each of the ewes: (1) one day before SMB implants were inserted, (2) Day 20 after PMSG was injected and (3) Day 40 after mating following ultrasound pregnancy test.

After all sera samples were collected for the year, they were assayed for PMSG antibodies in a single assay. Difference between binding of radioiodinated PMSG in 50 ml of sera with and without 250 ng unlabeled PMSG was used to quantitate presence of PMSG antibodies. Binding of PMSG to antibody provided by Dr. A.F. Parlow (Lot #61141575; 7600 CPM bound to 1:20,000 dilution) was used as positive control for binding in all assays. Difference in CPMS bound in sera from pregnant ewes vs. open ewes were tested for significance by general linear models procedures (SAS, 1989).

Induction of out-of-season breeding with SMB implants and PMSG injection was consistent over the 3-year treatment period. Percentage of ewes that were pregnant during years 1, 2 and 3 were 49.1, 48.0 and 45.0%, respectively.

Presence of antibodies in sera of ewes that were pregnant or open after repeated injections of PMSG are presented in Table 1.

Between open and pregnant ewes, CPM bound in 50 ml of sera were similar ($P > 0.05$). Of all the ewes treated with PMSG, four exhibited sera that bound $> 4,000$ CPM. Two of the four ewes were pregnant. These data indicate that failure of ewes to become pregnant after repeated injections of PMSG to induce out of season breeding is not due to immunoneutralization of PMSG.

The usefulness of embryo transfer programs are dependent upon an effective superovulation treatment (Holtz and Schlieper, 1991; Kummer et al., 1980). To induce out of season breeding (Evans and Robinson, 1980), a consistent response is needed before the technology can be adopted by commercial producers. Attempts to limit the biological activity of PMSG as a supplemental ovulatory tool by administering antisera to PMSG has yielded unsatisfactory results in enhancing pregnancy rates (Holtz and Schlieper, 1991; Kirkwood and Thacker, 1994). Injection of anti-PMSG in cattle did result in greater corpora lutea and fewer unovulated follicles (Gonzalez et al., 1994) or an improvement in quality of embryos (Saumande et al., 1984).

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Variable superovulatory responses may be related to production of PMSG antibodies in animals that are repeatedly injected with PMSG (Jainudeen et al., 1966; Youngs, 1992). In 1965, Hafez et al. evoked a response when cows were treated with PMSG the first and second times, but not of the third and fourth injection. Previously, it had been reported that various gonadotrophins evoked a progressive decline in ovarian response up to six successive ovulations (Willet et al., 1953). When dairy cows were given various treatments using PMSG and prostaglandin at seven to nine week intervals, a poor superovulatory response was observed (Saumande and Chupin, 1977). Superovulation of 14 heifers five to 10 times using PMSG and prostaglandin in a standardized regime at an interval of approximately 42 days indicated that a significant fall in near ovulation rate occurred between the first and second treatment but not between the second and subsequent treatments (Christie et al., 1979). When normal cows and problem breeder cows were injected repeatedly with PMSG, the proportion of viable embryos was higher for normal cows, but there was no effect of repeated injections in either normal or problem cows (Moor et al., 1984). In each of the above studies, sera were not examined for presence of PMSG antibodies.

Even though immunoneutralization has been speculated to be the reason for infertility in ewes bred out of season or for superovulated cows to become refractory to PMSG (Youngs, 1992), it is unlikely to be the source of the problem. To purposefully generate usable titers of antibody for radioimmunoassay or immunocytochemistry, adjuvants are used to enhance the changes for an immunobiologically response. In addition, booster injections are given to amplify the production of antibodies. Certainly, it is unlikely a yearly injection of PMSG to ewes without adju-

vant would be able to challenge the immune system sufficiently to generate great quantities of antibodies and evoke an effective immunoneutralization.

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Table 1. Presence of PMSG antibodies in ewe sera.

Year	Trt.	No. of Ewes	Repro. Status (N)	Time of bleeding ^a CPMS/50 µl sera			SEM
				1	2	3	
2	PMS	75	Preg (36)	951	577	502	168
			Open (39)	424	442	215	92
	Control	15	Open (15)	217	333	265	60
3	PMS	40	Preg (18)	648	554	492	71
			Open (22)	468	544	396	68
	Control	9	Open (9)	288	625	417	81

^a 1 = Sera obtained the day before SMB implants inserted.

2 = Sera obtained on Day 20 after PMS injection.

3 = Sera obtained on Day 40 after mating during ultrasound pregnancy test.