Serum Progesterone Concentrations Associated with Superovulation and Premature Corpus Luteum Failure in Dairy Goats

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ABSTRACT

The incidence and cause of premature corpora lutea failure and the response to luteinizing hormone treatment was investigated in superovulated dairy goats. Does were treated with 1000 IU pregnant mare serum gonadotropin intramuscularly, followed by either luteinizing hormone (treated group) or saline (control group). Serum progesterone concentrations were used to monitor corpus luteum function. The dose of pregnant mare serum gonadotropin used induced superovulation in a majority of the does, but the responses varied depending on the time of year. Premature regression of the corpora lutea occurred in 4 of 18 does after pregnant mare serum gonadotropin treatment, but there was no difference in the incidence of corpora lutea failure between treated and control groups. Decreases in serum progesterone concentrations were evident by day 3 after ovulation in does that experienced corpora lutea failure indicating this to be the critical time for premature regression of the corpora lutea in superovulated does.

Key words: Caprine, superovulation, corpora lutea, progesterone.

RÉSUMÉ

Cette expérience visait à étudier l'incidence et la cause de la régression prématurée des corps jaunes, ainsi que la réponse à l'injection de lutéinostimuline, chez des chèvres soumises à la superovulation. Elles reçurent d'abord une injection intramusculaire de 1000 I.U. de gonadotrophine équine; les sujets expérimentaux reçurent ensuite de la lutéinostimuline, tandis que les témoins ne reçurent que de la saline. La dose précitée de gonadotrophine équine provoqua une superovulation, chez la majorité des chèvres, mais leur réponse varia selon la période de l'année. La régression prématurée des corps jaunes se produisit chez quatre des 18 chèvres auxquelles on avait injecté de la gonadotrophine équine, mais on n'enregistra pas de différence dans l'incidence de la régression des corps jaunes, entre les chèvres expérimentales et les témoins. On constata une baisse de la teneur sérique en progestérone aussi tôt que trois jours après l'ovulation, indice que ce moment corespondrait à la période critique pour la régression des corps jaunes, chez les chèvres soumises à la superovulation.

Mots clés : chèvres, superovulation, corps jaunes, progestérone.

INTRODUCTION

Even though embryo transfer has been successfully carried out in goats (1,2) the technique is not sufficiently refined and the success of the procedure can be decreased by the occurrence of species specific problems (3). One specific problem in caprine embryo transfer is the premature regression of newly formed multiple corpora lutea in superovulated does (4,5,6,7,8,9). Embryo transfers in domestic ruminants are most successful when embryos are collected and transferred into recipients on day 6 or 7 postestrus. At this time in the does most blastocysts can be found in the uterus (10). Therefore surgical or nonsurgical uterine collection of embryos should ideally be carried out about one week after breeding. Premature regression of the corpora lutea and the occurrence of estrus before this time would result in the loss of embryos. There is a need for a better understanding of the phenomenon of premature regression of the corpora lutea in superovulated does, in order to formulate preventive measures. The objectives of this study were to determine the temporal factors associated with premature corpora lutea failure and associated early return to estrus in does following superovulatory treatments.

MATERIALS AND METHODS

Fourteen mature dairy does of various breeds were used in these studies. These consisted of eight does (five Alpine and three Saanen) purchased from a local farm and six

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Reprints will not be available.

This study was supported by a grant from the Ontario Ministry of Agriculture and Food.

Submitted May 23, 1985.

does of Alpine and Alpine X Saanen breeding from the Ontario Veterinary College (OVC) herd. In an attempt to extend normal estrous cycle activity into the expected transition period and anestrous season, does were exposed to a similar controlled lighting schedule as previously described (11) between January 1 and March 23, 1982. All goats were fed a ration of good quality hay, *ad libitum*, with supplementary grain feeding twice daily.

In experiment I, conducted between February 5 and March 5 four does of the OVC herd were used. In experiment II conducted between April 27 and May 23 four OVC and five purchased does were used and in experiment III conducted between May 27 and June 21 five OVC and seven purchased does were used.

In each trial the does were treated with a standardized program of hormone administration. Cloprostenol (175 μg Estrumate, ICI Pharma, Mississauga) was administered intramuscularly on day 0 followed 15 (experiment I) or 13 (experiment II and III) days later by 1000 IU of pregnant mare serum gonadotropin (PMSG), im (Equinex, Ayerst Laboratories, Montreal) to induce superovulation. Forty-eight hours after the PMSG injection, a second cloprostenol treatment (175 μg, im) was given and the does were randomly assigned to one of two groups. Goats in the control groups were treated with 1 mL saline sodium chloride (sc) every 48 h commencing two days after the second prostaglandin injection for four treatments. Goats in the treatment groups received 5 mg of pituitary luteinizing hormone (LH), (P.L.H., Schering, Pointe Claire, Quebec) diluted in 1 mL of saline sc, using the same schedule as for controls.

Three intact males were used on a rotating basis for the detection of estrus. All positive (E) and suspicious (e) indications of estrus were recorded. For the purpose of this study does that stood for mounting by the teaser male were recorded as showing estrus (E). Suspicious estrus (e) was recorded when a doe did not stand for the buck but exhibited bleating, tail wagging, squatting and urination in the presence of the buck.

Where possible jugular vein blood samples were collected from each doe on alternate days after the initial prostaglandin injection and then daily after PMSG was administered for twelve days. Samples were immediately refrigerated, later centrifuged and sera collected and stored at -20° C until assayed.

The progesterone (P_4) concentrations were determined using a radioimmunoassay method previously described and validated for goats (12). Duplicate aliquots of 0.2 or 0.4 mL of serum were used for extraction.

RESULTS

The source and distribution of does used in each of the trials, the ovulatory response and the fate of the corpora lutea are shown in Table I. In experiment I all four does showed estrus (E) at the expected time after prostaglandin injection. All does ovulated based on the serum progesterone patterns following PMSG treatment (Fig. 1). The progesterone patterns of one doe in each group (306 and 314) illustrated premature regression of the corpora lutea.

Does that maintained the corpora lutea exhibited a dramatic increase in P_4 concentrations during the luteal phase following superovulation and the high concentrations were maintained throughout the observation period. In does which suffered early corpora lutea failure a short lived increase in the progesterone concentrations occurred, followed by dramatic decreases to basal values (less than 1 ng per mL) within five to six days.

In experiment II, all the does were considered to have ovulated on the basis of the increased progesterone concentrations following the estrus induced by PMSG and prostaglandin treatment. However, premature cor-

TABLE I. Effect of LH and Saline Treatment Following Superovulation with 1000 IU PMSG in Dairy Goats

Doe	Treatment	Response to PMSG	Status of Corpora Lutea
Experiment I (82/2/5 to 82/3/5)			
287	LH	+	Maintained
306	LH	+	Failed
304	Saline	+	Maintained
314	Saline	+	Failed
Experiment II (82/4/27 to 82/5/23)			
184	LH	+	Maintained
195	LH	+	Maintained
206	LH	+	Maintained
314	LH	+	Maintained
326	LH	+	Failed
75	Saline	+	Maintained
182	Saline	+	Failed
287	Saline	+	Maintained
306	Saline	+	Maintained
Experiment III (82/5/27 to 82/6/21)			
75	LH	+	Maintained
181	LH	+	Maintained
182	LH	?	?
306	LH	—	—
56	LH	—	—
287	LH	—	—
314	Saline	+	Maintained
301	Saline	+	Maintained
109	Saline	+	Maintained
184	Saline	—	—
195	Saline	—	—
326	Saline	—	—

— = Does whose progesterone profile indicated their failure to respond to PMSG
? = Whether ovulation occurred in this doe is difficult to interpret from the progesterone profile and therefore has been excluded in the analysis

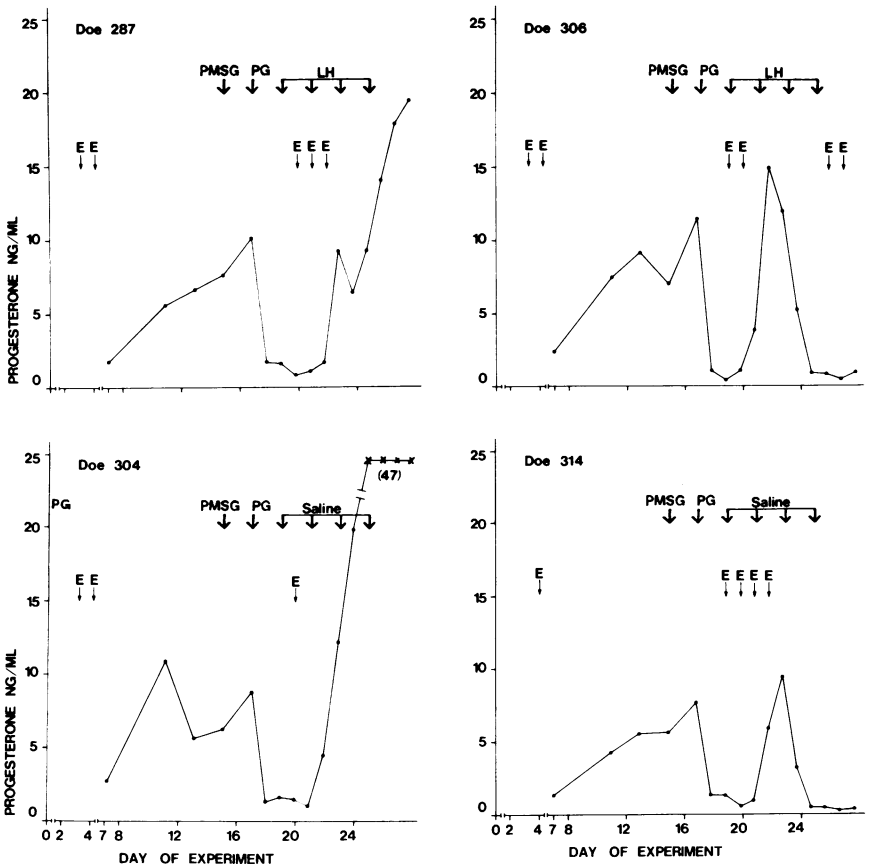

Fig. 1. Serum progesterone concentrations in does injected with LH or saline following PMSG superovulatory treatment during the normal breeding season. Upper arrows indicate injections with cloprostenol (PG), PMSG, LH or saline. E = standing for buck; e = other signs of estrus without actually standing. Initial cloprostenol treatment was administered on day 0.

pora lutea failure occurred in one of five goats treated with LH and one of four treated with saline (Table I). Serum progesterone profiles in animals with normal luteal function of premature luteolysis were similar to those illustrated in Figure 1.

In experiment III, eight animals showed signs of estrus (E: 109, 181, 195, 287, 301; e: 56, 306, 326) after the first cloprostenol injection but none of these exhibited a subsequent increase in P_4. Two other does (75 and 182) which did not exhibit estrous signs had elevated P_4 concentrations between the initial cloprostenol treatment and administration of PMSG. Nine of the 12 animals used in this trial exhibited estrus behaviour (E: 56, 109, 182, 184, 287, 301, 314; e: 195, 326) after the PMSG treatment but only four had subsequent increases in P_4. Two other does (75 and 181) did not show estrus after PMSG treatment but ovulated and formed functional corpora lutea as evidenced by increased P_4 concentrations.

Based on the serum P_4 patterns only five of the 12 does were considered to have ovulated after the superovulatory treatment (Figs. 2 and 3). Positive response to PMSG was indicated by progesterone concentrations rising above 1 ng per mL. All the does that ovulated, (75, 181 in the LH group and 109, 301, 314 in the saline group) were considered to have maintained the corpora lutea (Table I). One doe (182 in the LH group) displayed a progesterone pattern suggestive of shortlived poorly functioning corpora lutea which failed almost immediately or a partially luteinized follicle(s). Three animals in each treatment group did not exhibit a P_4 increase and therefore were classified acyclic. Three of these does (56, 184, 287) showed prolonged periods of sexual activity.

DISCUSSION

Since the optimum period for successful embryo collection after superovulation is the sixth or seventh day after mating or insemination, the corpora lutea must be maintained for at least six days for successful collection. In the present study the criteria for evaluating ovarian activity were: a) corpora lutea were considered to have been maintained if the serum P_4 concentrations increased above 1 ng per mL and remained elevated following the estrus induced by PMSG and cloprostenol treatment until day 6; or b) corpora lutea were considered to have failed prematurely if the serum P_4 concentrations showed a transient increase followed by a dramatic decrease below 1 ng per mL during the interval from estrus until day 6.

Based on these criteria, corpora lutea failure occurred in two of nine does which were treated with LH and two of nine does which were treated with saline (Table I). Thus there appears to be no difference in the incidence of corpora lutea failure between LH and saline treated does. Although there are several reports on the occurrence of corpora lutea failure in superovulated Angora goats (4,13) and feral does (8,9,14), this is the first documentation of the phenomenon in dairy does.

Premature regression of corpora lutea may occur as a consequence of rapid follicular development and ovulation of follicles before the granulosa cells have acquired the maturity necessary for optimal luteinization in response to the LH surge (9). It has also been postulated that the prolonged action of PMSG in the postovulatory period may be involved. In experiment III the prolonged period of sexual activity exhibited by three acyclic does suggests that the PMSG injection may have initiated follicular activity resulting in ovarian cysts. However, treatment of does with anti-PMSG has not reduce the incidence of luteal failure, and the phenomenon also occurs in does following treatment with a superovulatory dose of FSH (8). Finally, others have speculated that premature luteolysis is induced by endogenous prostaglandin arising

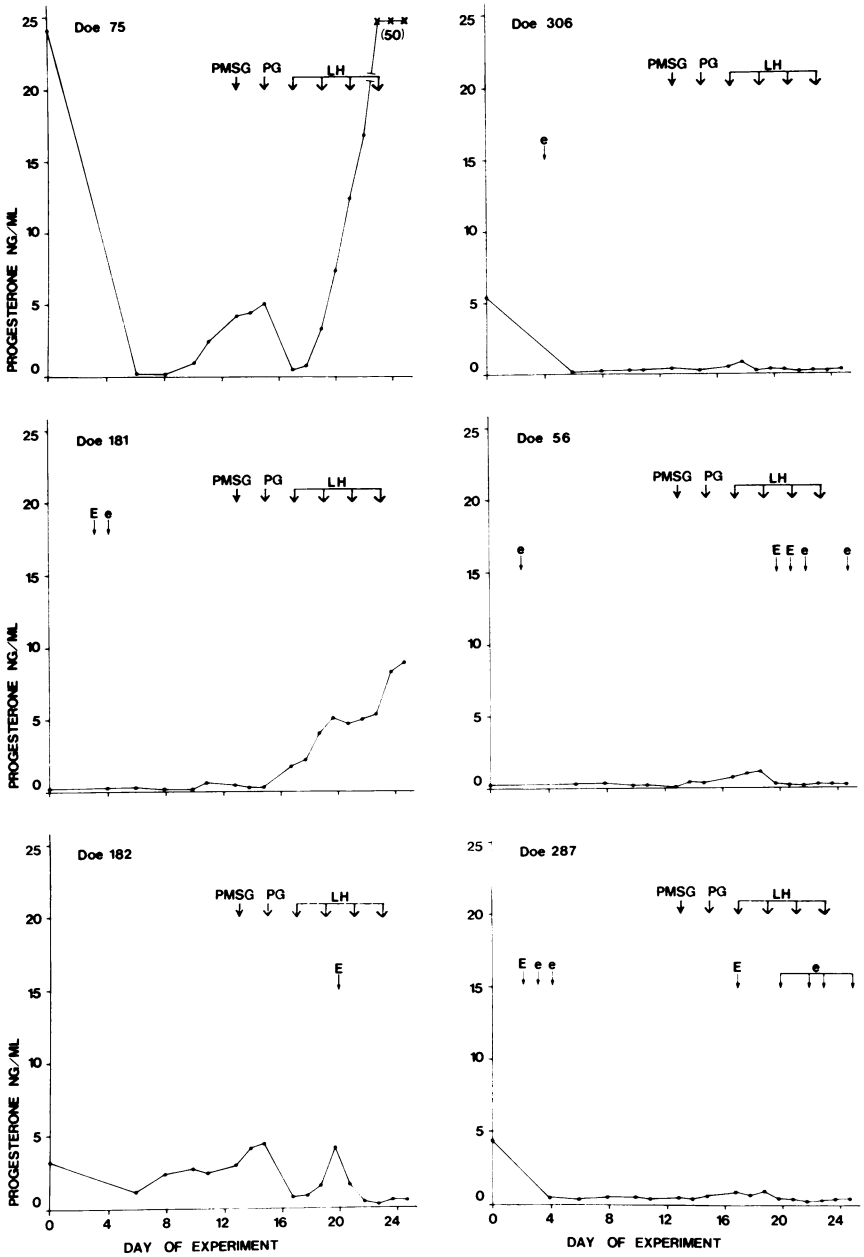

Fig. 2. Serum progesterone concentrations in does injected with LH following PMSG superovulatory treatment during the normal anestrous season. Initial cloprostenol treatment was administered on day 0.

from unovulated follicles or the uterus in response to prolonged stimulation by elevated levels of estradiol (14).

Camp *et al* (15) demonstrated that abnormally developing corpora lutea were more prevalent in does when the ovulation rate was greater than three, and that the sizes of the corpora lutea were related to the number present. These authors doubted the ability of smaller corpora lutea to respond to luteotrophic stimuli. They also proposed that impaired corpora lutea development in the goat might result from inadequate gonadotrophin stimulation prior to ovulation. Preliminary work by these authors has indicated considerably smaller spikes in LH concentrations associated with short estrous cycles compared to LH spikes associated with normal cycles.

In the present study, exogenous LH treatment did not prevent the occurrence of corpora lutea regression. Failure of LH treatment in this study might have been due to inadequate dosage, improper timing of treatment, or infrequent treatment or as suspected by others (15) the inability of the newly formed corpora lutea to respond to gonadotrophic stimuli. Further studies are necessary to determine the amount of LH needed, the proper timing of treatment and the frequency of treatment needed to prevent corpora lutea failure in superovulated does.

The occurrence of premature corpora lutea failure may be related to breed, since the incidence in this study (4/18) was lower than reported for Angora (13) and feral does (8). The time of year may have influenced the results. In this study does had been exposed to controlled lighting through late winter and early spring with the expectation that this would maintain ovarian activity through late spring. However, as indicated by the poor correlation between estrous behaviour and progesterone profiles in experiment III, normal cyclic pattern was not maintained in all cases. The normal progression of estrous activity from breeding (experiment I) to transition (experiment II) and finally to anestrus season (experiment III) was not significantly altered. Most researchers have previously reported on luteal failure in does superovulated during the breeding season (13,14). In the present study luteal failure occurred in does superovulated during the breeding season (2/4) and the period of transition to anestrus period (2/9), but not in the does superovulated during the seasonally anestrus period. Many of the does in experiment III were anestrus and few responded to the PMSG injections. Pretreatment with progesterone sponges may have improved the response in animals outside their normal breeding season (9).

There have been no reports regarding the critical period for premature regression of corpora lutea in goats. Armstrong *et al* (8,14) described the P_4 profile for does that have experienced corpora lutea failure, but the frequency and period of blood sampling were inadequate to determine the critical time of luteal failure. In the present study, does that experienced corpora lutea failure showed increas-

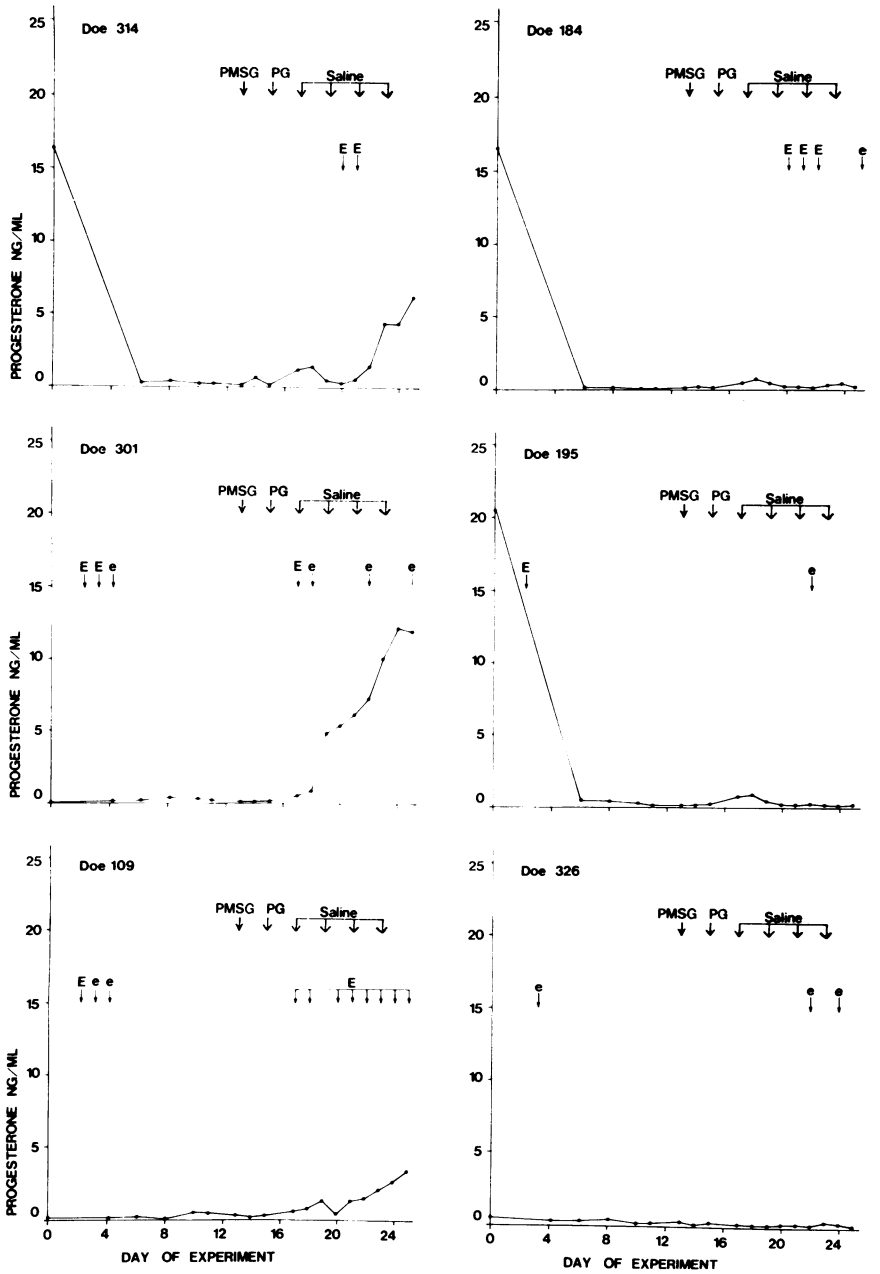

Fig. 3. Serum progesterone concentrations in does injected with saline following PMSG superovulatory treatment during the normal anestrous season. Initial cloprostenol treatment was administered on day 0.

ing P_4 values for a few days followed by a decline until concentrations were lower than 1 ng per mL by day 6. Thus, there was little difference in initial P_4 profiles between the two groups of does (corpora lutea maintained or failed) until day 3 after presumed ovulation. It was apparent, therefore, that the events leading to premature corpora lutea regression were initiated after luteal cells had formed and commenced synthesis and secretion of substantial quantities of P_4. This corroborates the gross appearance of premature regressed corpora lutea identified at day 6 by others (8).

REFERENCES

1. **CONSIDINE H.** Wisconsin veterinarian sparks first successful commercial ova transplant in dairy goats. Dairy Goat J 1980; 58: 3.
2. **COLFAX G.** Embryo transplants: the breeder's dream come true? Dairy Goat Guide 1982: 14-16.
3. **ARMSTRONG DT, EVANS G.** Factors influencing success of embryo transfer in sheep and goats. Theriogenology 1983; 19: 31-40.
4. **TERVIT HR, GOOLD PC, McKENZIE RD, CLARKSON DJ.** Embryo transfer in Angora goats. J Anim Sci (Suppl 1) 1980: 333.
5. **WARNES GM, PFITZNER AP, ARMSTRONG DT.** Embryo transfer procedures in the goat: factors which have a major influence on success rate. In: Shelton J, Trounson AO, Moore NW, eds. Embryo transfer in cattle, sheep and goats. Sydney: Aust Soc Reprod Biol 1982: 44-46.
6. **EPPLESTON J.** Embryo transfer procedure in the goat: physiological and procedural differences in superovulation and transfer between sheep and goats. In: Shelton J, Trounson AO, Moore NW, eds. Embryo transfer in cattle, sheep and goats. Sydney: Aust Soc Reprod Biol 1982: 41-43.
7. **ARMSTRONG DT, PFITZNER AP, WARNES GM, SEAMARK RF.** Factors affecting embryo survival following superovulation and embryo transfer in goats. Biol Reprod (Suppl 1) 1981: 110A.
8. **ARMSTRONG DT, MILLER BG, WALTON EA, PFITZNER AP, WARNES GM.** Endocrine response and factors which limit the response of follicles to PMSG and FSH. In: Shelton J, Trounson AO, Moore NW, eds. Embryo transfer in cattle, sheep and goats. Sydney: Aust Soc Reprod Biol 1982: 8-15.
9. **ARMSTRONG DT, PFITZNER AP, PORTER KJ, WARNES GM, JANSON PO, SEAMARK RF.** Ovarian responses of anoestrous goats to stimulation with pregnant mare serum gonadotrophin. Anim Reprod Sci 1982; 5: 15-23.
10. **HARRISON RJ.** The changes occurring in the ovary of the goat during the estrous cycle and in early pregnancy. J Anat 1948; 82: 21-47.
11. **ASHBROOK PF.** Year-round breeding for uniform milk production. In: Proc of the 3rd Int Conf on Goat Prod and Dis., Tucson 1982: 153-154.
12. **BOSU WTK, BARKER CAV.** Steroid levels after intramuscular injection of testosterone propionate in the caprine. Can J Comp Med 1982; 46: 390-394.
13. **ARMSTRONG DT, PFITZNER AP, WARNES GM, SEAMARK RF.** Superovulation treatments and embryo transfer in Angora goats. J Reprod Fert 1983; 67: 403-410.
14. **ARMSTRONG DT, PFITZNER AP, WARNES GM, RALPH MM, SEAMARK RF.** Endocrine responses of goats after induction of superovulation with PMSG and FSH. J Reprod Fert 1983; 67: 395-401.
15. **CAMP JC, WILDT DE, HOWARD PK, STUARD LD, CHAKRABORTY PK.** Ovarian activity during normal and abnormal length estrous cycles in the goat. Biol Reprod 1983; 28: 673-681.