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Unilateral Luteotropic Effect of Uterine Venous Effluent of a Gravid Uterine Horn in Sheep[1] (38988)

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In several species, including sheep, the uterus causes regression of the corpus luteum (CL), in the absence of pregnancy, through a direct or local pathway between the uterine horn and the adjacent ovary. The local pathway by which the uterine luteolysin passes between a uterine horn and adjacent ovary in sheep is venoarterial in nature and has been recently reviewed (1).

The mechanism by which an embryo overcomes the effect of the uterine luteolysin is unresolved. Ova transferred to an isolated (surgically separated) uterine horn, contralateral to the CL, did not prevent luteal regression, whereas ova transferred to the ipsilateral horn did (2). In ewes with CL in both ovaries, surgical separation of the uterine horns and transfer of fertilized ova to one horn resulted in luteal maintenance on the gravid side and luteal regression on the opposite, nongravid side. The life span of the CL in sheep, therefore, is regulated primarily by the relative positions of the nongravid horn and the CL. It was considered that the most probable mode of the luteotropic action of the embryo involves a local effect upon the endometrium by counteracting or preventing the release of the uterine luteolysin (2, 3). The possibility that the embryo or gravid horn may produce a luteotropin that is transported directly from the gravid horn to the adjacent CL has not been ruled out, however.

The present experiment tested the hypothesis that the embryo or gravid uterine horn in sheep secretes a blood-borne luteotropin into the main uterine vein which unilaterally inhibits the effect of the uterine luteolysin.

Materials and methods. Twenty-four mature, crossbred ewes (Columbia, Suffolk and Targe) were used. Ewes were observed twice daily for estrus using vasectomized rams. When a ewe stood for mounting, estrus was recorded and the day was designated as Day 0 of the estrous cycle. All ewes were mated at least twice using two fertile rams, beginning 12 hr after the onset of estrus and were mated twice every 12 hr thereafter as long as ewes stood for mounting. On Day 5, ewes were laparotomized midventrally under halothane anaesthesia and CL were marked with India ink as described (4). Only ewes with at least one CL in each ovary were used. In all such ewes the uterine horns were separated by severing the intercornual ligament to the point of internal bifurcation of the uterus. A silk ligature was placed around one horn at the bifurcation (side selected at random) and the horn was transected cranial to the ligature, to produce an isolated uterine horn. Hemostasis was maintained through minimal use of electrocautery. The caudal (transected) end of the isolated horn was left open to the abdominal cavity which prevented the establishment of pregnancy in the transected horn but not in the opposite, intact horn. In this manner, a gravid and a nongravid horn were established in bilaterally ovulating ewes. Ewes were randomized into three groups. Group 1 ewes served as controls. In Group 2 ewes, the main uterine vein (uterine branch of uteroovarian vein) from the gravid side was freed, transected and surgically anastomosed, end to side, to the corresponding vein on the nongravid side (Fig. 1). An end-to-end anastomosis was done if more than one major branch existed on the recipient side. In Group 3

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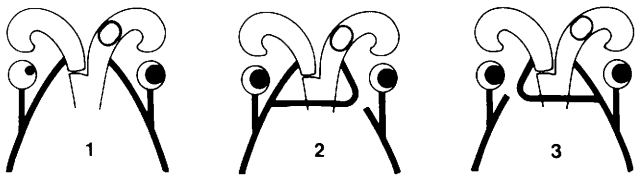

CONTROL (5)[a]		VENOUS ANASTOMOSIS: gravid to nongravid side (3)		nongravid to gravid side (3)	
Nongravid	Gravid	Nongravid	Gravid	Nongravid	Gravid
Mean (mg) ± SE	Mean (mg) ± SE	Mean (mg) ± SE	Mean (mg) ± SE	Mean (mg) ± SE	Mean (mg) ± SE
136[b] ± 22	615[c] ± 48	471[c] ± 24	530[c] ± 26	490[c] ± 41	500[c] ± 120

FIG. 1. Mean corpus luteum (CL) weights for bilaterally ovulating, unilaterally pregnant ewes as effected by surgical anastomosis of uterine veins. Surgery was done on Day 5 of diestrus and necropsies were done on Day 20. In all ewes, the uterine horns were surgically separated and one was ligated and transected at the internal bifurcation to produce a nongravid horn and a gravid horn. Group 1 served as controls. In the other two groups a surgical anastomosis of the main uterine veins was done (end to side) from gravid to nongravid side (Group 2) or from nongravid to gravid side (Group 3).[a] Number of ewes in each group are in parentheses. bc, mean CL weights with different superscripts are different ($P < .01$).

ewes, the reciprocal anastomosis was done (anastomosis of the main uterine vein from nongravid to gravid side). Standard vascular surgical techniques were used as described (5). Patency of the anastomosis was checked by observation for free flow. Sodium heparin solution was given iv (10,000 units) to all ewes with anastomosed veins immediately before transection of the donor vein and sc every 12 hr for 36 hr beginning immediately after surgery. Penicillin and streptomycin were given once daily for 4 days.

Ewes were observed daily for estrus and necropsied on Day 20 (15 days postsurgically). The marked CL were removed, weighed and classified as maintained, partially regressed or regressed as described (6). The uterine horns were examined for the absence of fluid accumulation or embryos on the isolated side and the presence of an intact (nonfragmented and apparently viable) embryo on the uterine-intact side. The surgically anastomosed veins were examined for patency by gently flushing saline through the uterine vascular system and observing flow through the anastomosis. Patency was arbitrarily classified from 0 (occluded) to 4 (unimpeded flow). Only ewes with an intact embryo (all groups) and those with a surgical anastomosis with patency classified as 3 or 4 (Groups 2 and 3) were used.

Weights of CL were statistically analyzed by analysis of variance with a hierarchical classification and mean CL weights were compared by the Waller–Duncan multiple range test (7).

Results. Eleven ewes (five in group 1, three in group 2, and three in group 3) had an intact embryo on Day 20 (all groups) and a patent surgical anastomosis (Groups 2 and 3). The interaction of group and side was significant ($P < .005$) and seemed due primarily to small weight of CL on the nongravid side in control ewes (Fig. 1). Based on the multiple-range test, mean CL weight was less, ($P < .01$) on the nongravid side in the control ewes (all CL classified as regressed), than on the gravid side in control ewes or for either side in the other two groups (all CL classified as maintained). There were no significant differences among the mean weights of CL on the gravid side in control ewes and either side in the other two groups.

Discussion. The presence of a nongravid uterine horn (produced by isolation and transection of the horn) in bilaterally ovulating ewes resulted in luteal maintenance on the gravid side and luteal regression on the

nongravid side in all five ewes which were pregnant. These results confirm the findings of a unilateral association between the location of the embryo and maintenance of the CL (2).

It is most likely that the unilateral luteal regression of CL on the side of the nongravid horn in unilaterally pregnant sheep is exerted through a venoarterial pathway as has been demonstrated in nonpregnant sheep (6, 8). The pathway involves discharge of the luteolysin into the uterine venous effluent and direct transfer to the ipsilateral ovarian artery (9, 10). The results of the present experiment indicate that the uterine luteolysin in the venous effluent from the nongravid horn was rendered ineffective when the effluent also contained venous blood from a gravid horn. This occurred whether the surgical anastomosis of the main uterine vein was from gravid to nongravid side (Fig. 1, Group 2) or from nongravid to gravid side (Group 3). These results support the hypothesis that the unilateral luteotropic effect of the embryo involves a blood-borne luteotropin which exerts its effect outside of the uterine horn rather than at the level of the endometrium.

Maintenance of CL on the nongravid side in Group 3 can be attributed to passage of the luteolysin, through the surgical anastomosis, to the gravid side. A similar result was obtained in unilaterally hysterectomized, nonpregnant sheep with surgical anastomosis of uterine veins (6, 8); the CL on the intact side was maintained, whereas the CL on the unilaterally hysterectomized side regressed when the uterine venous effluent from the intact uterine horn was diverted to the hysterectomized side.

Thirteen ewes were eliminated because of absence of an intact embryo on Day 20, or occlusion or partial occlusion of the surgical anastomosis. Absence of an embryo (four, one, and two ewes in Groups 1, 2 and 3, respectively) resulted in luteal regression or partial regression (based on appearance and size of CL) on both sides. This result suggests that surgical trauma associated with the anastomosis procedure did not, in itself, contribute to luteal maintenance in Groups 2 and 3. In pregnant ewes with an occluded anastomosis (four ewes in Group 2 and two in Group 3) regression or partial regression of CL occurred on the nongravid side and maintenance occurred on the gravid side, which was similar to the results in the pregnant, control ewes.

Prostaglandin (PG) $F_{2\alpha}$ has been shown to have potent luteolytic properties and is considered by many to be the uterine luteolysin in ewes (10, 11). Secretion of $PGF_{2\alpha}$ by the sheep uterus increases just prior to and during luteal regression (12, 13). Paradoxically, $PGF_{2\alpha}$ has been found to be present in high levels in the uterine venous effluent, not only during the time of luteal regression in cycling ewes, but also during the corresponding days of pregnancy when the CL is being maintained (14, 15). Diekman and Niswender found greater variation in $PGF_{2\alpha}$ levels in the uterine venous blood of cycling ewes and suggested that fewer $PGF_{2\alpha}$ peaks in pregnant ewes may contribute to maintenance of the CL (16). The present results are compatible with the hypothesis that $PGF_{2\alpha}$ is the luteolysin even though high levels of $PGF_{2\alpha}$ have been found in the uterine venous blood in pregnant ewes. The CL regressed when the ipsilateral uterine vein contained blood from only the nongravid horn, indicating the presence of uterine luteolysin, whereas CL were maintained when the ipsilateral uterine vein contained venous blood from a gravid horn, whether or not it also contained blood from a nongravid horn. Maintenance of CL could be attributed to dilution of the uterine venous effluent from the nongravid horn by blood from the gravid horn, but this would likely have resulted in at least partial regression of the Cl.

The present results indicate that the main uterine vein is involved in the unilateral luteotropic effect of a gravid horn and raises the question whether the luteotropin, like the uterine luteolysin, passes from gravid horn to adjacent ovary through a local venoarterial pathway. However, the present study was not designed to determine whether the site of action of the luteotropin was within the vascular system or within the

ovary. In this regard, it has been reported that there was no apparent difference between pregnant and nonpregnant ewes in transfer of $PGF_{2\alpha}$ to the ovarian artery on Day 15 after ovulation (15), nor was there any difference between pregnant and nonpregnant ewes in mean uptake by CL of tritiated $PGF_{2\alpha}$ infused into the ovarian artery (17). Inskeep and Pexton injected $PGF_{2\alpha}$ into the CL-bearing ovary in pregnant and nonpregnant ewes (18). Although progesterone levels did not appear to be different between groups, 5 of 8 pregnant ewes and 4 of 23 nonpregnant ewes did not return to estrus. It was suggested that an embryonic luteotropin reduces the sensitivity of sheep CL to $PGF_{2\alpha}$.

Placental extracts have been shown to have luteotropic activity in the guinea pig (19), hamster (20), rat (21), mouse (22) and more recently, in the ewe (3) and in gilts (23). In pregnant rabbits, plasma progesterone has been found to be significantly higher than in pseudopregnant rabbits on Day 5 after ovulation, indicating the probability of a luteotropic stimulus prior to implantation (24). Morishige and Rothchild reported that a placental luteotropin in the rat apparently replaced pituitary prolactin in maintaining luteal progesterone production after Day 7 of pregnancy (25). Linkie and Niswender characterized the rat placental luteotropin in peripheral blood on Day 12 of pregnancy as a heat-labile protein of approximately 25,000–50,000 mol wt (26). In the rhesus monkey, a surge in progesterone secretion was noted between Days 9 and 11 of gestation (27). More recently, human chorionic gonadotropin (HCG) has been demonstrated in peripheral serum on Day 8 of gestation (28) and it is likely that HCG is luteotropic in the human female. It has, therefore, been demonstrated that in a number of species the presence of a fertilized blastocyst in the uterus prevents luteal regression and in some cases results in an increased production of progesterone by the CL. The results of the present experiment, however, provide apparently the first direct evidence in any species for the secretion of a blood-borne luteotropin by a gravid uterine horn or its contents, which unilaterally inhibits the effect of the uterine luteolysin.

Summary. The involvement of the main uterine vein in the unilateral maintenance of CL was studied in bilaterally ovulating, unilaterally pregnant ewes. Ewes were mated at estrus (Day 0) and bilaterally ovulating ewes were randomized into three groups at surgery on Day 5. In all ewes, the uterine horns were separated through the intercornual area and one was ligated and transected near the internal bifurcation to produce a nongravid horn. One group served as controls (five ewes). In the other two groups the main uterine vein on one side was surgically anastomosed (end to side) to the corresponding vein of the opposite side (gravid side to nongravid side in one group—three ewes, and nongravid side to gravid side in the other—three ewes). Necropsies were done on Day 20. Mean CL weight was less, ($P < .01$) on the nongravid side in control ewes than on the gravid side in control ewes or for either side in the other two groups. There were no significant differences among mean weights of CL on the gravid side in control ewes and either side in the other two groups. The CL regressed when the ipsilateral uterine vein contained blood from only the nongravid horn whereas the CL was maintained when the ipsilateral uterine vein contained venous blood from a gravid horn, whether or not it also contained blood from a nongravid horn. Results indicate that the uterine venous effluent from a gravid uterine horn in sheep has a luteotropic effect on the ipsilateral CL.

1. Ginther, O. J., J. Anim. Sci. **39,** 550 (1974).
2. Moor, R. M., and Rowson, L. E. A., J. Reprod. Fert. **12,** 539 (1966).
3. Rowson, L. E. A., and Moor, R. M., J. Reprod. Fert. **13,** 511 (1967).
4. Ginther, O. J., Endocrinology **83,** 613 (1968).
5. Ginther, O. J., and Bisgard, G. E., Amer. J. Vet. Res. **33,** 1583 (1972).
6. Mapletoft, R. J., and Ginther, O. J., Amer. J. Vet. Res. **36,** 957 (1975).
7. Waller, R. A., and Duncan, D. B., J. Amer. Stat. Assoc. **64,** 1484 (1969).
8. Ginther, O. J., Del Campo, C. H., and Rawlings C. A., Amer. J. Vet. Res. **34,** 723 (1973).
9. McCracken, J. A., Baird, D. T., and Goding, J. R., Recent Prog. Hormone Res. **27,** 537 (1971).

10. McCracken, J. A., Carlson, J. C., Glew, M. E., Goding, J. R., and Baird, D. T., Nature New Biol. **238,** 129 (1972).
11. Goding, J. R., Cumming, I. A., Chamley, W. A., Brown, J. M., Cain, M. D., Cerini, J. C., Cerini, M. E. D., Findlay, J. K., O'Shea, J. D., and Pemberton, D. H., Gynecol. Invest. **2,** 73 (1971-72).
12. Wilson, L., Jr., Cenedella, R. J., Butcher, R. L., and Inskeep, E. K., J. Anim. Sci. **34,** 93 (1972).
13. Barcikowski, B., Carlson, J. C., Wilson, L., and McCracken, J. A., Endocrinology **95,** 1340 (1974).
14. Wilson, L., Jr., Butcher, R. L., and Inskeep, E. K., Prostaglandins **1,** 479 (1972).
15. Pexton, J. E., Weems, C. W., Smutny, W. J., Pitts, R. E., and Inskeep, E. K., J. Anim. Sci. **39,** 221 (Abstract) (1974).
16. Diekman, M. Z., and Niswender, G. D., Proceedings of the 7th Annual Meeting of the Society for the Study of Reproduction, Ottawa, Canada, pp. 162, (Abstract No. 166) (1974).
17. Staigmiller, R. B., and Ellinwood, W. E., Proceedings of the 7th Annual Meeting of the Society for the Study of Reproduction, Ottawa, Canada, pp. 55, (Abstract No. 40) (1974).
18. Inskeep, E. K., and Pexton, J. E., J. Anim. Sci. **39,** 212 (Abstract) (1974).
19. Macht, D. I., Stickers, A. E., and Seckinger, D. L., Amer. J. Physiol. **88,** 65 (1929).
20. Klein, M., Proc. R. Soc. B, **125,** 348 (1938).
21. Ray, E. W., Averill, S. C., Lyons, W. R., and Johnson, R. W., Endocrinology **56,** 359 (1955).
22. Cerruti, R. A., and Lyons, W. R., Endocrinology **67,** 884 (1960).
23. Longenecker, D. E., and Day, B. N., J. Reprod. Fert. **31,** 171 (1972).
24. Fuchs, A. R., and Beling, C., Endocrinology **95,** 1054 (1954).
25. Morishige, W. K., and Rothchild, I., Endocrinology **95,** 260 (1974).
26. Linkie, D. M., and Niswender, G. D., Biol. Reprod. **8,** 48 (1973).
27. Neill, J. D., Johansson, E. D. B., and Knobil, E.. Endocrinology **84,** 45 (1969).
28. Mishell, D. R., Jr., Nakamura, R. M., Barberia, J. M., and Thorneycroft, I. H., Amer. J. Obstet, Gynecol. **118,** 990 (1974).

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