PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 153, 289-294 (1976)

# Local Venoarterial Pathway for Uterine-Induced Luteolysis in Cows[1] (39530)

R. J. MAPLETOFT,[2] M. R. DEL CAMPO,[2] AND O. J. GINTHER

*Department of Veterinary Science, University of Wisconsin, Madison, Wisconsin 53706*

In the cow, as in the ewe, there exists a local uteroovarian pathway for uterine-induced luteolysis. Removal of one uterine horn results in luteal maintenance when the corpus luteum (CL) is on the unilaterally hysterectomized side, but not when the CL is on the uterine-intact side (1, 2). Similarly, sectioning the broad ligament between the uterine horn and adjacent ovary also results in cycle prolongation when a CL exists in that ovary (3). In addition, the luteolytic effect of oxytocin (1), progesterone (4), and an intra-uterine plastic coil (5) have been shown to be locally mediated, acting directly between uterine horn and adjacent ovary.

In the ewe, the local pathway by which the uterine luteolysin passes between the uterine horn and adjacent ovary is venoarterial in nature, involving veins which drain the uterine horn and the ipsilateral ovarian artery (2). Although the mechanism by which the luteolysin passes from vein into artery is unresolved, passage is likely favored by the close contact between veins which contain uterine venous blood and the adjacent ovarian artery. The ovarian artery in sheep is tortuous and closely applied to the wall of the uteroovarian (ovarian) vein (6, 7). The local uteroovarian venoarterial pathway for uterine-induced luteolysis in ewes was demonstrated by experiments involving surgical anastomosis of vessels in the uteroovarian vascular pedicle (8, 9, 10). Unilateral hysterectomy adjacent to the ovary bearing the CL resulted in luteal maintenance, however, surgical anastomosis of either the main uterine vein or the ovarian branch of the ovarian artery from intact side to the corresponding vessel on the hysterectomized side resulted in luteal regression on the hysterectomized side. Results therefore demonstrated, that in the ewe, a local venoarterial pathway (involving main uterine vein and adjacent ovarian artery) operated in the physiologic process of uterine-induced luteal regression.

In the cow, a common uteroovarian (ovarian) vein drains the ovary, uterine tube and much of the uterine horn and the ovarian artery is very tortuous and closely applied to the surface of the uteroovarian vein (11, 12; Fig. 1). The similarity of vascular anatomy of the uteroovarian pedicle in the ewe and cow therefore, led to the hypothesis that the local uteroovarian pathway for uterine-induced luteolysis in the cow is also venoarterial in nature (11). The purpose of the present experiment was to determine whether the main uterine vein and ovarian artery were involved in the local uteroovarian luteolytic pathway in cows.

*Materials and Methods.* Mature Holstein cows were observed twice daily for estrus and the day that a cow stood for mounting was designated day 0 of the estrous cycle. Ovulation and CL formation and development were confirmed by rectal palpation. Cows were fasted for 48 hr and surgery was done on days 8, 9, 10 or 11 of the cycle. Anesthesia was maintained with halothane. Mid-ventral laparotomy was done and CL were marked in all cows with India Ink. After surgery, cows were observed twice daily for estrus and were necropsied on day 30 (9 days after the expected return to estrus).

Cows were allotted to groups 1-4 at surgery and group 5 was added at necrospy (Fig. 2). In all cows a unilateral hysterectomy was done (contralateral to CL in group 1 and ipsilateral to CL in all other groups) and the cut edge of the broad ligament on

[1] Research supported by the College of Agricultural and Life Sciences and Grant No. 630-0505B from the Ford Foundation. The authors thank Drs. W. J. Tyler and E. R. Hauser for animals.

[2] Postdoctoral Trainees, Endocrinology-Reproductive Physiology Training Program, University of Wisconsin, Madison. R. J. Mapletoft is supported by the Medical Research Council of Canada.

Fig. 1 is used herein for illustrative purposes and was first published in Am. J. Vet. Res. 35, 193 (1974).

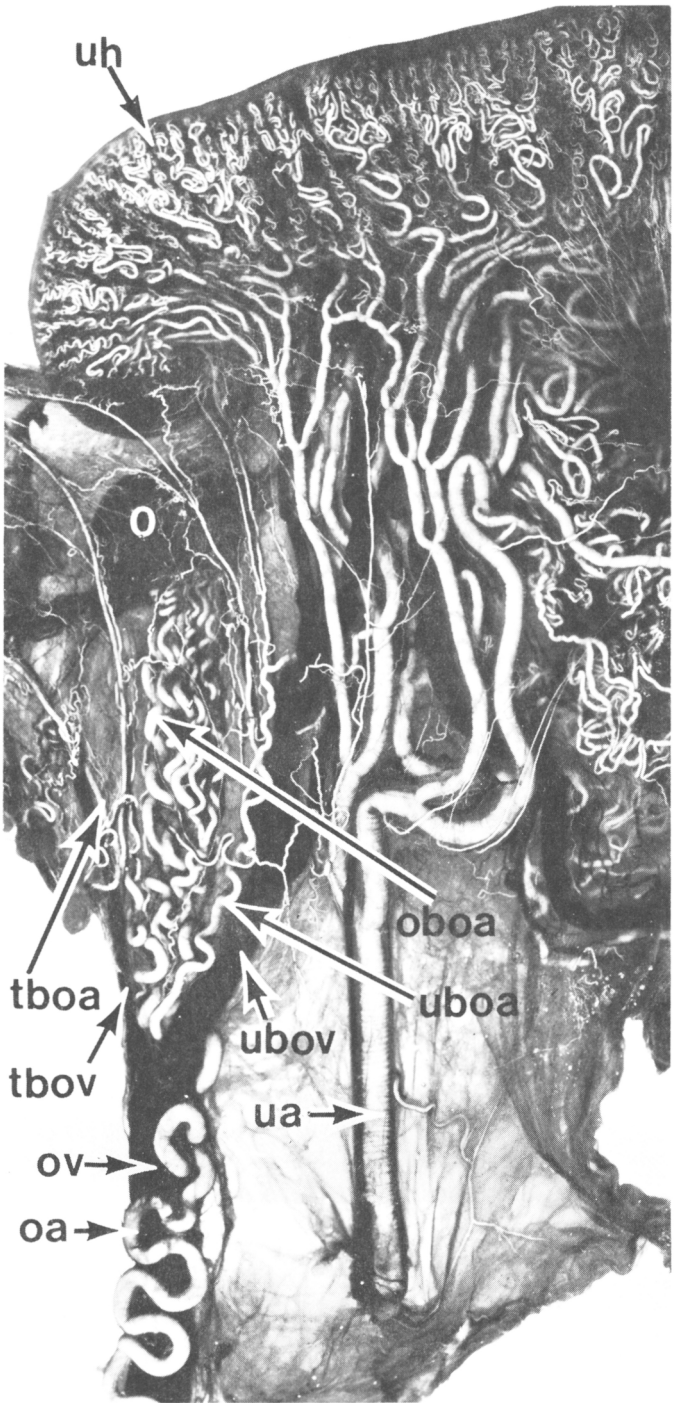


FIG. 1. Dorsal view of the left uteroovarian vascular pedicle in a heifer. Veins (dark) and arteries (light) were injected and tissues were fixed and cleared. A common uteroovarian (ovarian) vein drains the ovary, uterine tube, and much of the uterine horn. The ovarian artery is very tortuous and is closely applied to the uteroovarian vein. Terminally, the ovarian artery divides into a uterine branch and an ovarian branch and the latter divides into a tubal branch and numerous ovarian branches which enter the ovary. It is proposed that the local luteolytic effect of the uterus in cows is exerted through a venoarterial pathway which involves discharge of the uterine luteolysin into veins which drain the uterine horn (main uterine vein and uteroovarian vein) and transfer into the adjacent ovarian artery which carries the luteolysin to the ovary. O = ovary, uh = uterine horn, oa = ovarian artery; ov = ovarian (uteroovarian) vein; ua = uterine artery; oboa = ovarian branches of ovarian artery; tboa = tubal branch of ovarian artery; tbov = tubal branch of ovarian vein; uboa = uterine branch of ovarian artery; ubov = uterine branch of ovarian vein (main uterine vein).

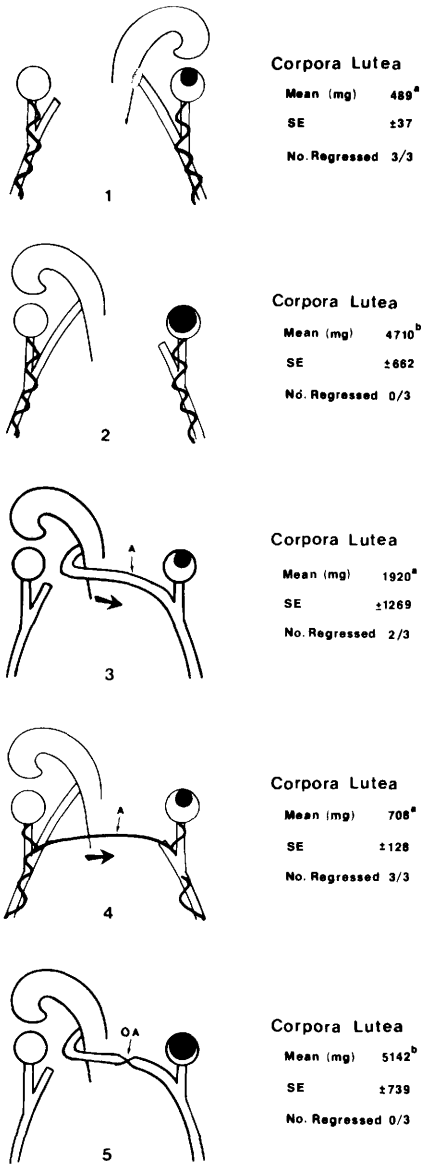


FIG. 2. Effect on the corpus luteum in cows of surgical anastomosis (A) of the main uterine vein (group 3) or the uterine branch of the ovarian artery (group 4) from uterine-intact side to hysterectomized side. The venous anastomosis in group 5 was occluded (OA). Surgery was done on days 8–11 of diestrus and necropsies were done on day 30. All cows were unilaterally hysterectomized, ipsilateral (group 1), and contralateral (groups 2–5) to the CL and surgical anastomosis of the main uterine vein (groups 3 and 5) and of the uterine branch of the ovarian artery (group 4) was done. The arrow in groups 3 and 4 indicates the direction of blood flow. Mean CL weights with different superscripts are significantly different ($P < 0.05$).

the hysterectomized side was sutured to the intact uterine horn, as described (1). In group 3, the main uterine vein (uterine branch of the ovarian vein) on the uterine-intact side was surgically anastomosed to the corresponding vein on the hysterectomized side. An end-to-end venous anastomosis was done with 6-0 cardiovascular silk using standard vascular surgical techniques (8). Patency of anastomosis was checked by observation for free flow and the adjacent serosa was sutured over the anastomosis to give it additional support. In group 4, the uterine branch of the ovarian artery on the uterine-intact side (donor artery) was surgically anastomosed to the uterine branch of the ovarian artery on the hysterectomized side (recipient artery). The technique for freeing the arteries and performing an end-to-end anastomosis has been described (9). Upon completion of the anastomosis, the ovarian artery on the recipient side was ligated 3 times at levels approaching the aorta to minimize the development of collateral vessels so that the ovary on that side would be supplied only through the surgical anastomosis. Patency of the surgical anastomosis was indicated by distension of the recipient artery and maintenance of color of the CL in the recipient ovary. Sodium heparin solution was given iv (40,000 units) to cows in groups 3 and 4 immediately after removal of the uterine horn and every 12 hr for 36 hr, beginning immediately after surgery. Penicillin and streptomycin were given twice daily for 3 days.

At necropsy on day 30, marked CL were removed, weighed and classified as regressed (small, white, firm and encapsulated), maintained (large, soft and flesh colored), or partially regressed (intermediate size, color and consistency). In addition, ovaries were examined for follicular development and evidence of recent ovulations. The surgically anastomosed vessels were examined for patency by gently flushing saline into the uterine artery (group 3) or ovarian artery (group 4) on the donor side. Patency was indicated by venous discharge of saline on the recipient side in group 3 and oozing of saline from the site of the removed CL in group 4. Cows in group 3 with an occluded venous anastomosis were replaced and allot-

ted to group 5, whereas cows in group 4 with an occluded arterial anastomosis were replaced.

Weights of CL were statistically analyzed by a one-way analysis of variance and mean CL weights were compared by the protected lsd test for multiple comparisons (significant F value for treatment effect in the analysis of variance).

*Results.* At necropsy, 3 cows had an occluded venous anastomosis and 1 cow had an occluded arterial anastomosis. Based on multiple comparisons (Fig. 2), mean weight of CL was less ($P < 0.05$) in group 1 controls with unilateral hysterectomy contralateral to CL (489 mg; CL regressed in 3 of 3 cows), in group 3 with unilateral hysterectomy ipsilateral to the CL and surgical anastomosis of the main uterine vein from intact side to hysterectomized side (1920 mg; CL regressed in 2 of 3 cows) and in group 4 with unilateral hysterectomy ipsilateral to CL and surgical anastomosis of uterine branch of ovarian artery from intact to hysterectomized side (708 mg; CL regressed in 3 of 3 cows) than in group 2 controls with unilateral hysterectomy ipsilateral to CL (4710 mg; CL maintained in 3 of 3 cows) and in group 5 with unilateral hysterectomy ipsilateral to CL and occluded venous anastomosis (5142 mg; CL maintained in 3 of 3 cows). The mean weight of CL was not significantly different among groups 1, 3 and 4 or between groups 2 and 5.

*Discussion.* Removal of the uterine horn on the side opposite to the ovary bearing the CL appeared to have no effect on the degree of luteal regression by day 30 suggesting that surgical operation on days 8 to 11 of diestrus did not in itself interfere with luteal regression. This was not, however, tested critically, since unoperated controls were not included. Removal of the uterine horn on the side ipsilateral to the CL resulted in luteal maintenance confirming the involvement of a local or unilateral uteroovarian pathway for uterine-induced luteolysis in cows.

Surgical anastomosis of the main uterine vein from intact side to hysterectomized side (Fig. 2, group 3) resulted in luteal regression in 2 of 3 cows indicating that the uterine luetolysin was being delivered to the ovarian vascular pedicle on the hysterectomized side. In the cow in which luteal regression did not occur it was noted at necropsy that the surgically anastomosed veins were twisted by tension on the broad ligament. Saline could only be flushed through the surgical anastomosis when the serosa over the veins was dissected away. Cows in group 5 (occluded venous anastomosis with thrombus formation) provide additional support for the hypothesis that the main uterine vein serves as the uterine or initial component of the pathway. Luteal maintenance occurred in 3 of 3 cows indicating that a functional (patent) vein is necessary to deliver the luteolysin to the site of venoarterial transfer (presumably in the ovarian vascular pedicle).

When the ovarian arterial blood from the uterine-intact side supplied the CL on the unilaterally hysterectomized side luteal regression occurred (Fig. 2, group 4) indicating that the ovarian artery delivered the uterine luteolysin to the ovary through the surgical anastomosis. This result indicates that the ovarian artery serves as the distal or ovarian component of the local uteroovarian pathway for uterine-induced luteolysis in cows. Presumably, venoarterial transfer of uterine luteolysin occurred on the uterine-intact side in areas of close contact between veins containing uterine venous blood and the ovarian artery. The uterine branch of the ovarian artery was used for the surgical anastomosis because the ovarian branches of the ovarian artery were too small and too inaccessible for surgical anastomosis. Ligations of the proximal segment of the recipient ovarian artery facilitated flow through the anastomosis and minimized the probability that the recipient ovary would be supplied by collateral vessels which would contain no luteolysin. Indeed, upon completion of the surgical anastomosis and ligations on the recipient side, the vessels distended with blood and the CL maintained its pink color, indicating that it was being supplied by arterial blood.

In cattle there exists a prominent uteroovarian arterial anastomosis between a branch of the uterine artery and the uterine branch of the ovarian artery (11, 12). It appeared to be quite dynamic, being significantly

more prominent on the side of the CL and therefore apparently changes in diameter, depending on the side of ovulation (11). The physiologic role of the difference between the two sides in the diameter of the anastomosis and the direction of blood flow in the anastomosis are not known. However, it has been suggested that the uteroovarian arterial anastomosis is necessary for normal cyclic ovarian function (12, 13). Surgical anastomosis of the main uterine vein from intact to hysterectomized side resulted in luteal regression on the hysterectomized side. This result tends to rule out the necessity of the uteroovarian arterial anastomosis for luteal regression in the cow (Fig. 2).

In the cow in which the arterial anastomosis had become occluded, partial luteal regression occurred (2219 mg). Perhaps a restricted ovarian arterial blood flow can induce at least partial luteal regression in the cow, as has been observed in the ewe (14). This could be a suggested mode of luteal regression in group 4, however, available evidence tends to rule this possibility out. In ewes in which the blood flow to the ovary had been compromised, not only CL regression but also ovarian inactivity and atrophy occurred (14). Luteal regression occurred in group 4 cows with no apparent alteration in ovarian activity. Although a control anastomosis was not done in control cows (group 2), transection and reanastomosis of the ovarian artery in unilaterally hysterectomized ewes did not cause any evidence of luteal regression (10). Furthermore, the ovary on the recipient side in group 4 cows was not deprived of arterial blood during the anastomosis procedure. When the proximal segment of the recipient ovarian artery was ligated, the CL maintained its pink color indicating that it continued to receive arterial blood (presumably through the surgical anastomosis). Present results in the cow are therefore consistent with those reported previously in the ewe (9, 10). Although estrous cycle length was not analyzed, there did not appear to be any difference between cows with surgical anastomosis (groups 3 and 4) and group 1 controls. Obviously, studies are required to determine critically the adequacy of the venoarterial pathway for complete luteolysis.

Results, therefore, indicate that the main uterine vein serves as an initial or uterine component and that the ovarian artery serves as a distal or ovarian component of the local uteroovarian pathway for uterine-induced luteolysis in the cow. Results are consistent with the hypothesis that the uterus regulates the lifespan of the CL in cows through a local venoarterial pathway.

*Summary.* The involvement of the main uterine vein and ovarian artery in the local pathway for uterine-induced luteolysis was studied in mature Holstein cows. At surgery on days 8–11 of diestrus, all cows were unilaterally hysterectomized and allocated to one of 4 groups: 1) controls with hysterectomy contralateral to CL, 2) controls with hysterectomy ipsilateral to CL, 3) hysterectomy ipsilateral to CL and surgical anastomosis of the main uterine vein from intact to hysterectomized side, and 4) hysterectomy ipsilateral to CL and surgical anastomosis of ovarian artery from intact to hysterectomized side. At necropsy on day 30, the surgical anastomosis was examined for patency and CL were weighed. Cows with an occluded venous anastomosis were reassigned to group 5. CL weights from 15 cows (3 per group) were analyzed. Mean weight of CL was less ($P < 0.05$) for group 1 controls (489 mg), group 3 with venous anastomosis (1920 mg), and group 4 with arterial anastomosis (708 mg), than for group 2 controls (4710 mg) or group 5 with occluded venous anastomosis (5142 mg). Mean weight of CL was not significantly different among groups 1, 3, and 4 or between groups 2 and 5. Results indicate the involvement of a venoarterial pathway in the local uteroovarian luteolytic effect in cows. The main uterine vein serves as the proximal or uterine component and the ovarian artery serves as the distal or ovarian component of the pathway.

1. Ginther, O. J., Woody, C. O., Mahajan, S., Janakiraman, K., and Casida, L. E., J. Reprod. Fert. **14,** 225 (1967).
2. Ginther, O. J., J. Anim. Sci. **39,** 550 (1974).
3. Hixon, J. E., and Hansel, W., Biol. Reprod. **11,** 543 (1974).
4. Woody, C. O., and Ginther, O. J., J. Anim. Sci. **27,** 1387 (1968).
5. Ginther, O. J., Woody, C. O., Janakiraman, K.,

and Casida, L. E., J. Reprod. Fert. **12,** 193 (1966).
6. Del Campo, C. H., and Ginther, O. J., Am. J. Vet. Res. **34,** 1377 (1973).
7. Ginther, O. J., and Del Campo, C. H., Am. J. Vet. Res. **34,** 1387 (1973).
8. Ginther, O. J., and Bisgard, G. E., Am. J. Vet. Res. **33,** 1583 (1972).
9. Ginther, O. J., Del Campo, C. H., and Rawlings, C. A., Am. J. Vet. Res. **34,** 723 (1973).
10. Mapletoft, R. J., and Ginther, O. J., Am. J. Vet. Res. **36,** 957 (1975).
11. Ginther, O. J., and Del Campo, C. H., Am. J. Vet. Res. **35,** 193 (1974).
12. Lamond, D. R., and Drost, M., J. Anim. Sci. **38,** 106 (1974).
13. Lamond, D. R., Tomlinson, R. V., Drost, M., Hendricks, D. M., and Jöchle, W., Prostaglandins **4,** 269 (1973).
14. Mapletoft, R. J., Lapin, D. R., and Ginther, O. J., Biol. Reprod. **15,** 414 (1976).

Received May 3, 1976. P.S.E.B.M. 1976, Vol. 153.