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Progesterone in Uterine and Arterial Tissue and in Jugular and Uterine Venous Plasma of Sheep[1]

C. W. WEEMS,[2] Y. S. WEEMS, C. N. LEE, and D. L. VINCENT

Department of Animal Sciences
University of Hawaii at Manoa
Honolulu, Hawaii 96822

ABSTRACT

Concentrations of progesterone in uterine and arterial tissue and in uterine and jugular venous plasma were determined. Blood was collected on Days 4 and 9 postestrus from the jugular vein and the first and last venous branches draining each uterine cornu; uterine tissue and arteries were subsequently collected. Progesterone was greater ($p<0.05$) in the cranial third than in the middle or caudal thirds of the uterine horn adjacent to the corpus luteum (CL)-bearing ovary or in any third of the contralateral horn. Progesterone in uterine arterial segments adjacent to the CL-bearing ovary was higher ($p<0.05$) than in contralateral segments. Progesterone was higher ($p<0.05$) in blood from the first venous branch of the cranial third of the uterine cornu adjacent to the ovary with the CL, than in the last branch of the caudal third, or contralateral horn, or in jugular blood. When oviductal veins were resected on Day 9 postestrus, progesterone in the first vein draining the cranial third of the uterine cornu adjacent to the CL-containing ovary was not different ($p>0.05$) 48 h after resection than in the same vessel in the opposite horn or in jugular blood. We concluded that progesterone and other ovarian products may be delivered to the uterus locally.

INTRODUCTION

Most of the economic losses in livestock are due to early embryonic death (Ayalon, 1978). The hormonal environment within or between uterine cornua may influence early embryonic development (Rowson et al., 1972). More successful pregnancies occur when bovine embryos are transferred to the uterine horn adjacent to the corpus luteum (CL) than when transferred to the uterine horn opposite the CL (Newcomb and Rowson, 1976; Sreenan, 1976; Christie et al., 1979; Newcomb et al., 1980). When bovine embryos are transferred to each uterine cornu, embryonic death is greater in the uterine horn opposite the CL (Del Campo et al., 1979).

Physiological and anatomical parameters are different between uterine and ovarian arteries ipsilateral or contralateral to the ovary with a CL (Del Campo and Ginther, 1973; Ginther, 1974; Ford et al., 1976, 1980). Differences in levels of progesterone or other ovarian secretory products delivered systemically or locally may explain why embryos of sheep remain predominantly in the uterine horn ipsilateral to the ovary with a single CL (Scanlon, 1972; Reimers et al., 1973).

Prostaglandins E_1 and E_2, which are potent antiluteolytic agents, have been demonstrated to be effective in maintaining luteal function only when infused in the uterine cornu adjacent to the CL-bearing ovary in the ewe (Magness et al., 1981, Huie et al., 1981; Weems et al., 1985). However, maternal recognition of pregnancy may be more complex than was first thought (Heap et al., 1988). Survival of the embryo during early pregnancy is closely associated with progesterone and the timing of developmental changes in the uterine environment (Lawson and Cahill, 1983). Vincent et al. (1986) reported that progesterone can alter the uterine environment and advance the release of PGE_2 in the presence of an older embryo. Thus, routes of delivery, time of delivery, and distribution of ovarian products to the uterus may determine whether pregnancy is successful. These studies were conducted to determine distribution of progesterone within the ovine uterus, uterine arterial tissue, and to compare progesterone in peripheral blood with concentrations of progesterone in uterine venous blood.

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MATERIALS AND METHODS

Animal and Surgical Procedures

Mature crossbred ewes were penned with brisket-painted vasectomized rams for detection of estrus. Marked ewes were removed from the flock twice daily (0600 and 1800 h) and estrus (Day 0) was confirmed with a separate vasectomized ram. Only ewes with 16- to 18-day estrous cycles were used. Ewes were anesthetized with Rompun (Bayvet Corp., Shawnee, KS), given a local anesthetic (Lidocaine, Sigma Chemical Co., St. Louis, MO) s.c. at the abdominal midline, and the reproductive tract was exteriorized through a midventral incision. Combiotic (Pfizer Inc., New York, NY) was given s.c. after the first surgery in Study 2. All blood samples were collected after anesthesia and surgery on ewes with a single CL.

Study 1

Twenty ewes were assigned randomly at estrus to be sampled on Day 4 or Day 9 postestrus (10 ewes/group). Blood was collected in random order, from the jugular vein from the first vein draining the cranial half of each uterine cornu, or from the last vein draining the caudal half of each uterine cornu. Blood samples (10 ml) were collected by venipuncture, plasma was collected by centrifugation, and plasma was stored frozen (–20°C) until assayed for progesterone.

After collection of blood, ewes were killed with an overdose of Rompun, and the reproductive tract and associated vasculature were excised. Segments of each uterine arterial branch, distal to the first bifurcation but before entry into the uterus, from each uterine cornu adjacent or opposite the ovary with the CL were collected. Arterial segments were split longitudinally, rinsed of blood with sterile physiological saline, and stored frozen until assayed for progesterone by radioimmunoassay (RIA). Uterine cornua were separated, disconnected from the cervix, and cleaned of mesometrium and extraneous vasculature. Each uterine cornu was divided into cranial, middle, and caudal thirds and stored frozen until assayed for progesterone. Progesterone in tissue and blood was quantified by RIA.

Study 2

Blood (10 ml) was collected, in random order, by venipuncture from the jugular vein and the first major uterine vein of the cranial half of each uterine cornu on Day 9 postestrus (0 h) and at 48 h after ligation and resection of a segment of each oviductal vein (10 ewes). Plasma was collected by centrifugation and stored frozen (–20°C) until assayed for progesterone.

RIA for Progesterone

Progesterone in plasma and tissues was extracted, chromatographed, and quantified by RIA as described previously (Magness et al., 1981), with the following modifications. Tissue samples were homogenized with radiolabeled progesterone added to each sample for estimation of extraction recoveries for progesterone. Plasma samples were extracted in triplicate, with one replicate of each sample receiving radiolabeled progesterone for estimation of recoveries for each set of assay duplicates. Tissue samples were extracted four times, and blood samples were extracted two times with freshly opened anesthesia-grade diethyl ether (Mallinkrodt, St. Louis, MO). Efficiency of extraction and chromatography were corrected for procedural losses. Within- and between-assay variations from pooled samples for plasma and uterine tissue and uterine artery after corrections for tissue weight were 3.2% and 6.9% for plasma; 7.7% and 10.1% for uterine tissue after correction for tissue weight; and 6.2% and 8.6% for uterine artery after correction for tissue weight.

Statistical Analysis

Data for concentrations of progesterone in plasma, uterine tissue, and uterine artery were analyzed by a factorial design for a completely randomized analysis of variance. Where significant effects were found, means were compared by a Student-Newman-Keul's Multiple Range Test (Steel and Torrie, 1960).

RESULTS

In Study 1, progesterone in each region of uterine tissue (Fig. 1), jugular or uterine venous sample (Fig. 2), or in uterine arterial tissue (Fig. 3) increased ($p<0.05$) from Day 4 to Day 9 postestrus. Concentrations of progesterone were greater ($p<0.05$) in the cranial than in the middle or caudal third of uterine tissue adjacent to the ovary with the CL on Day 4 or Day 9 postestrus (Fig. 1). In addition, levels of progesterone were greater ($p<0.05$) in the middle than in the caudal third of the uterine horn adjacent to the CL-bearing

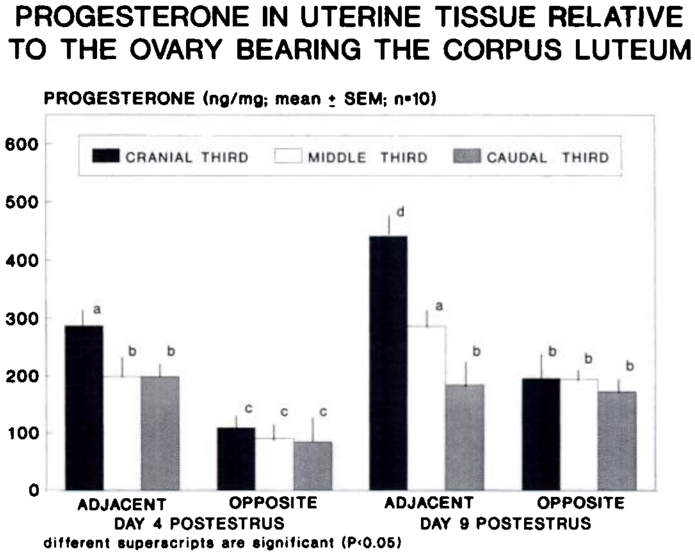

FIG. 1. Mean (+SEM) concentrations (ng/mg) of progesterone in uterine tissue in the cranial, middle, and caudal thirds of each uterine cornu, adjacent and opposite the ovary bearing the corpus luteum on Days 4 and 9 postestrus. *Bars* with different *superscripts* are significantly different ($p<0.05$).

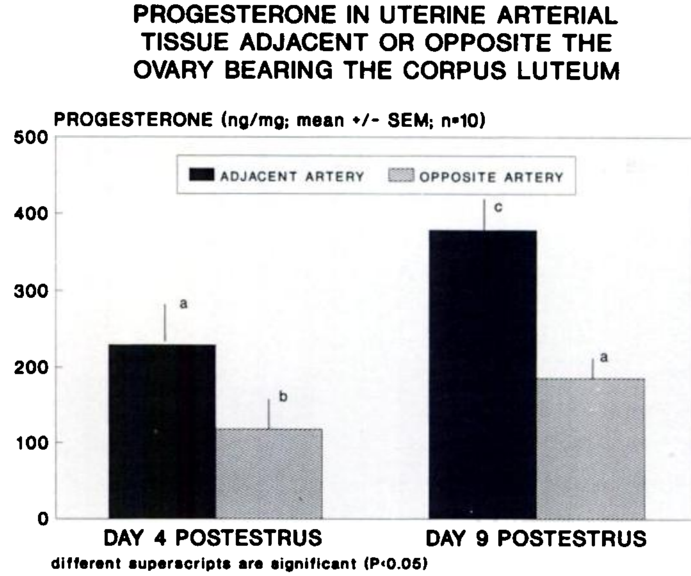

FIG. 3. Mean (+SEM) concentrations (ng/mg tissue) of progesterone in uterine arterial tissue adjacent to or opposite the ovary bearing the corpus luteum on Days 4 and 9 postestrus. *Bars* with different *superscripts* are significantly different ($p<0.05$).

ovary only on Day 9 postestrus (Fig. 1). However, concentrations of progesterone were not different ($p>0.05$) between regions of the uterine horn contralateral to the ovary with the CL or the caudal third of the uterine horn adjacent to the CL-bearing ovary, except on Day 4 postestrus ($p<0.05$) (Fig. 1). Concentrations of progesterone in the cranial or middle third of the uterine cornu adjacent to the CL-bearing ovary were greater ($p<0.05$) than in the same regions from the opposite uterine horn (Fig. 1).

Concentrations of progesterone in plasma from the first branch of the uterine vein proximal to the ovary and adjacent to the ovary bearing the CL were greater ($p<0.05$) than in the jugular vein or last branch of the uterine vein draining the caudal third of the uterine cornu (Fig. 2). In addition, concentrations of progesterone were greater ($p<0.05$) in the first uterine venous branch adjacent to the CL-bearing ovary than in the first or last uterine venous branches draining the contralateral uterine horn (Fig. 2). Levels of progesterone in the jugular vein or in the last vein draining either caudal third of each uterine cornu (Fig. 2) did not differ ($p>0.05$) on Days 4 or 9 postestrus. Concentrations of progesterone in uterine arterial tissue on Days 4 or 9 postestrus were greater ($p<0.05$) in tissue adjacent than in tissue opposite the CL-bearing ovary (Fig. 3).

In Study 2, concentrations of progesterone at 0 h on Day 9 postestrus in the first venous branch draining uterine tissue adjacent to the CL-bearing ovary were greater ($p<0.05$) than in samples collected from the same venous branch contralateral to the CL-bearing ovary or in jugular plasma (Fig. 4). However, 48 h after ligation and resection of the oviductal vein that origi-

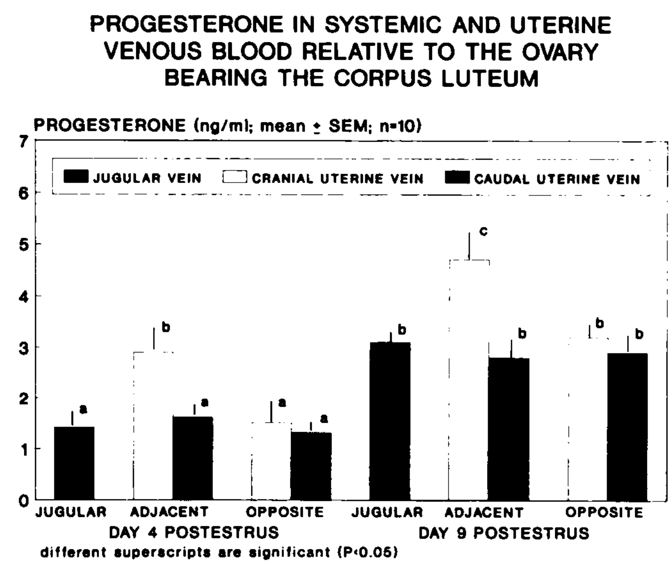

FIG. 2. Mean (+SEM) concentrations (ng/ml) of progesterone in the jugular vein and in the cranial and caudal uterine veins adjacent and opposite the ovary bearing the corpus luteum on Days 4 and 9 postestrus. *Bars* with different *superscripts* are significantly different ($p<0.05$).

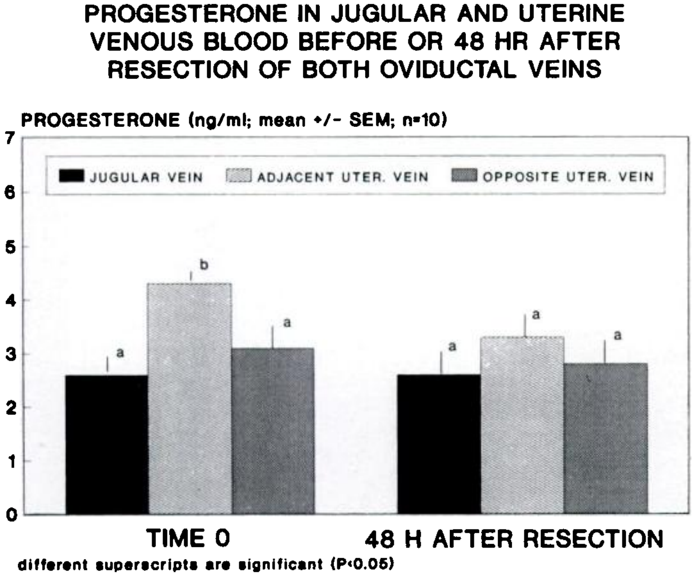

FIG. 4. Mean (+SEM) concentrations (ng/ml) of progesterone in the jugular vein and adjacent and opposite uterine veins before (Time 0) and 48 h after ligation and resection of both oviductal veins. *Bars* with different *superscripts* are significantly different (p<0.05).

nates near the ovary and usually anastomoses with the first venous branch draining the cranial portion of the uterine cornu, concentrations of progesterone were not different (p>0.05) in samples collected from the jugular vein or the first venous branch draining uterine tissue adjacent or opposite the ovary with the CL (Fig. 4).

DISCUSSION

Data in Figures 1 and 3 clearly indicate that concentrations of progesterone are not the same within regions of the uterine cornu adjacent to the side of ovulation, between uterine cornu, or in the uterine arterial tissue adjacent to or opposite the ovary containing the CL of sheep. These data are in agreement with similar data reported in cattle (Pope et al., 1982; Weems et al., 1988). In addition, concentrations of progesterone are not the same in veins draining different regions within or between uterine horns in sheep reported herein or in cattle (Weems et al., 1988). These data may explain why embryos are influenced to remain predominantly in the uterine horn adjacent to the side of ovulation in unilaterally ovulating ewes (Scanlon, 1972) and why embryonic death is greater opposite the side of ovulation in cows when embryos are transferred into each uterine horn (Del Campo et al., 1979). Likewise, unilateral ovariectomy arrests development and decreases implantation in the uterine horn ipsilateral to the side of the hemiovariectomy of pregnant rats, a species with multiple ovulations on each ovary (Nuti et al., 1971). Therefore, an adjacent ovary appears to provide a more favorable environment for implantation and embryonic survival through local delivery of ovarian products to the ipsilateral uterine cornu.

The oviductal vein adjacent to the CL-containing ovary is a possible pathway for local delivery of progesterone or other ovarian products to the first venous branch draining the cranial portion of the uterine horn. Concentrations of progesterone are greater in the uterine branch vein draining the cranial than in the vein draining the caudal portion of the uterine horn or in jugular blood. However, concentrations of progesterone in these same veins are not different 48 h after resection of the oviductal vein (Fig. 4). The oviductal vein originates around the ovary and usually continues as the first venous branch, draining the cranial uterine cornu (Del Campo and Ginther, 1973; Ginther and Del Campo, 1973). Staples et al. (1982) reported that progesterone was greater in uterine lymph adjacent to the CL-bearing ovary than in peripheral blood of sheep, unless an ovary with a CL was not adjacent to the side of cannulation. Also, they noticed marked anastomoses between uterine and ovarian lymphatic ducts but suggested that retrograde lymph flow from the ovary to the uterus was not possible. Therefore, regional differences in levels of progesterone in uterine tissue in sheep reported herein or in the cow could be from contributions of ovarian lymphatics and/or the adjacent oviductal vein.

Gradients of progesterone in uterine tissue adjacent to the ovary with the CL could be maintained by close apposition or coiling of branches of the uterine artery around veins draining the uterine tissue similar to that seen for the anatomical relationship between the uterine branch of the ovarian vein and the ovarian artery (Ginther, 1974). Similar mechanisms for concentrating progesterone in the ovarian artery of the CL-bearing ovary from the utero-ovarian vein have been reported for the ewe (Welsh et al., 1979). Therefore, these data clarify the unexplained observations of Ford et al. (1975), where uterine venous progesterone was greater than in jugular plasma.

Twenty to thirty percent of prenatal mortality of fertilized ova occurs during the first weeks of pregnancy (Edey, 1969). Variations in stage of embryonic development (Wilmut et al., 1985) and levels of maternal progesterone and overfeeding effects on levels of progesterone have been postulated to be related to lower embryonic survival in farm species (Parr et al., 1982). Reduction of progesterone by inhibitors of 3β-hydroxysteroid dehydrogenase (Ashworth et al., 1987) or by overfeeding (Williams and Cumming, 1982; Parr et al., 1987) decreases peripheral progesterone and pregnancy rates in sheep. Supplementation with exogenous progesterone has been reported to increase survival rate of embryos in intact sheep in some (Pearce et al., 1984; Davis et al., 1986) but not all studies (Parr et al., 1987). Progesterone replacement increases survival rates of embryos transferred into ovariectomized ewes in a dose-dependent manner (Parr et al., 1982). These variable effects of progesterone on survival rates of fertilized sheep ova may be related to differences in levels of progesterone in peripheral blood and those delivered locally as depicted in Figures 1–4.

These local effects of an ovary on an ipsilateral uterine horn may not always be positive. Ewe lambs have lower pregnancy rates than mature ewes (Stellflug et al. 1988). When the oviductal vein adjacent to the side of ovulation in ewe lambs was resected late on Day 4 or early Day 5 after breeding, pregnancy rate increased from 27.5% in control ewe lambs to 72.7% in ewe lambs with the oviductal vein resected. In addition, laparotomy of these ewe lambs at Day 85 of gestation revealed that all fetuses were ipsilateral to the side of ovulation in the group with the oviductal vein resected, whereas half of the fetuses were contralateral to the side of ovulation in control ewe lambs. Moreover, the higher pregnancy rate in ewe lambs with the oviductal vein resected was associated with lower peripheral progesterone during the period of maternal recognition of pregnancy but with higher overall levels of progesterone through Day 85 of gestation (Gemmer, 1987). It is concluded that progesterone and probably other ovarian products are delivered locally to a uterine cornu adjacent to the CL as well as through systemic vasculature, which may provide a more favorable environment for a successful pregnancy in the uterine curnu ipsilateral to an ovary with a CL.

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