

Adaptations to reduction of endometrial surface available for placental development in sheep

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Summary. On Day 5 of pregnancy, before the blastocyst migrates to the uterus, one uterine horn was ligated to restrict the trophoblast to the lumen ipsilateral to the corpus luteum. The numbers of placentomes (caruncles and cotyledons) were reduced by half, but neither at 120 nor at 140 days of pregnancy (term 147 days) did the weights of placentae and fetuses of treated ewes differ significantly from those of control ewes. Amongst uterus-ligated animals prepared for chronic study, the rate of uterine blood flow (electromagnetic flow transducer, ml/min) to the pregnant horn was higher than in control ewes, as was the concentration of progestagens in maternal peripheral blood. There may be a compensatory response that causes hypertrophy of placentomes and that increases blood flow to the uterine horn containing placental tissue.

Introduction

When one horn of the bipartite uterus of sheep was ligated early in pregnancy, the number of maternal caruncles available for placental development was reduced by half but there was no discernible effect on the weight of the fetus at either 120 or 140 days of pregnancy (term 145-150 days) (Bazer *et al.*, 1979). This raised two questions that we have sought to answer in the present experiments: (1) what are the components of the compensatory response and (2) what mechanisms bring them about?

Materials and Methods

Experiments were performed on 52 ewes of the Rambouillet or Florida native breed. On the 5th day after mating (Day 0), which is before the embryo has entered the uterus, 32 ewes were anaesthetized with oxygen and methoxyflurane (Metafane: Pitman-Moore, Washington Crossing, NJ). After a thorough scrub with povidone-iodine solution, the abdomen was opened with a midline incision to expose the reproductive tract. A large ligature (umbilical tape 6 mm in width) was used to occlude the lumen of one horn (Bazer *et al.*, 1979). After recovery from the operation, ewes were returned to the flock. No surgery was performed on animals serving as controls.

Acute experiments

On Days 120 and 140 of pregnancy, 20 control and 17 experimental ewes were anaesthetized with oxygen and methoxyflurane and placed in dorsolateral recumbency. The abdomen was opened with a midline incision to expose the reproductive tract. The uterus was excised intact and opened carefully to expose fetal membranes and to ensure that they had not passed into the ligated uterine horn. Free fluid in the ligated, non-gravid uterine horn was collected, the volume measured,

and an aliquant saved for analysis, results of which were included in an earlier study (Bazer *et al.*, 1979). Amniotic and allantoic fluids were collected separately and their volumes measured. The fetus was removed, dried thoroughly, and weighed. The crown-to-rump length was measured to compare with normal fetal growth curves to verify dates of pregnancy (Barcroft, 1964). Fetal and maternal components of the placenta were separated by squeezing each caruncle at the base. Cotyledons, the fetal portion of the placenta that interdigitates with maternal caruncles, were dissected from placental membranes and were counted and weighed. The intact placenta was also weighed, as was the uterus after all fetal tissue and tissues of the broad ligament, cervix, oviducts and ovaries had been trimmed away.

Chronic experiments

Between Days 80 and 110 of pregnancy, 15 ewes were brought from the barn to the laboratory where they were housed in pens (3 × 3 m) in a covered, screened enclosure and observed for 1–2 weeks to be sure that they were eating well and maintaining or gaining weight and that they appeared healthy. The day before surgery, they were deprived of food and water. The ewes were anaesthetized with oxygen and methoxyflurane and were placed in a left lateral recumbent position; the abdomen was opened with a midline incision. We identified the uterine horn that contained the fetus (5 ligated animals) or that was ipsilateral to the ovary that contained a corpus luteum (9 control ewes). A blood flow transducer was placed around a segment of the main artery serving that uterine horn after the point at which the artery diverged from the vaginal branch but before any major divisions in the broad ligament. Connective tissue around a segment of the artery was infiltrated with 3–5 ml 2% lignocaine (Lidocaine, Astra, Worcester, MA) to prevent spasm of the artery. The peritoneum was incised to place a flow transducer (4–6 mm) around the artery and was closed. The flow transducer was sutured in place and its free end brought out to a pouch fixed to the flank of the ewe. The free end of a polyvinyl catheter, placed in the femoral artery, was also brought to the pouch (Caton, Abrams, Clapp & Barron, 1974a).

Animals were allowed at least 4 days to recover from surgery. They were then brought into the laboratory in a wheeled cart each day at 08:00 h in the company of another ewe. They were allowed 1 h to adjust, then blood flow was measured and recorded continuously between 09:00 and 10:00 h (Narco RT 500 electromagnetic flow meter—DM5 recording apparatus, Houston, TX). Once blood flow was stable, blood was withdrawn from the arterial catheter with a Harvard pump at a constant rate (0.5 ml/min) for 20 min. Blood was centrifuged and the plasma frozen until analysed for concentrations of progesterone, oestrone and oestradiol by radioimmunoassay. Occasionally, problems with catheters or flow transducers precluded simultaneous measurements.

Rate of blood flow for a particular day and time was calculated by using the mean of 20 measurements taken from the continuous recording at 1-min intervals coincident with blood sampling. Standard deviation never exceeded 4% of the mean for any animal. Flow transducers were calibrated *in vitro* before and after placement in each animal (Roman-Ponce, Thatcher, Caton, Barron & Wilcox, 1978).

All animals delivered live lambs either spontaneously at term or by Caesarean section preterm in the event that catheters and flow transducers both stopped working. In each instance, fetal crown-to-rump length was appropriate for gestational age (Barcroft, 1964).

Hormone assays

Oestrone and oestradiol. Plasma (3–4 ml) was extracted twice with 2 volumes of anhydrous diethyl ether. Oestrone and oestradiol were isolated from the dried ether by column chromatography (Sephadex LH-20).

Antiserum to oestrone-6-carboxymethylxime-BSA was prepared in rabbits. After correction for endogenous plasma levels, recovery of 20, 50 and 100 pg oestrone added to ewe plasma was 24.1

± 1.9 , 51.6 ± 3.6 and 99.9 ± 5.9 pg (mean \pm s.d.), respectively (8 samples each). The sensitivity was 5 pg oestrone and the inter- and intra-assay coefficients of variation were 14.4 and 4.7%, respectively.

Antiserum to 6-keto-oestradiol-BSA was obtained from Dr G. D. Niswender, Colorado State University. When 20, 50 and 100 pg oestradiol were added to ewe plasma, recovery after correction for endogenous plasma levels was 18 ± 2.3 , 53.5 ± 3.5 and 100.6 ± 10.4 pg/ml, respectively (4 samples each). The sensitivity of the assay was 10 pg/tube and the inter- and intra-assay coefficients of variation were 15.5 and 6.2%, respectively (Caton, Wilcox & Kalra, 1980).

Progestagens. Progestagens were measured after extraction of 0.2–0.5 ml plasma with 2,2,4-trimethylpentane (iso-octane) without further chromatographic purification. Antiserum to 11α -hydroxyprogesterone hemisuccinate-BSA was a gift of Dr J. L. Fleegeer, Texas A&M University. This antiserum cross-reacts with other progestagens, principally 5α -pregnane-3,20-dione (20%) and 20α - and 20β -dihydroprogesterone (5%). Addition of 100, 300 and 500 pg progesterone to ewe plasma resulted in recovery of 103.6 ± 6.2 , 308.5 ± 16.7 and 531.0 ± 31.0 pg/tube, respectively (6 samples each) after correction for endogenous levels. Sensitivity of the assay was 20 pg/tube and inter- and intra-assay coefficients of variation were 12.6 and 4.8%, respectively. Validations of these hormone assays are given by Caton, Wilcox & Kalra (1980).

Statistical methods

Data from control and ligated animals were compared by using an analysis of variance (ANOVA). Effects of day of pregnancy (120 and 140 days), singleton and twin fetuses, and ligated and control uterine horns were evaluated by using a 2×2 factorial design. Appropriate orthogonal contrasts were used to detect differences amongst specific treatments. Data from chronic experiments were compared by calculating least square means by day of pregnancy and fitting data to the highest order polynomial equation (≤ 5) that was statistically significant. Curves were then compared by using a test for heterogeneity of regression.

Results

Acute experiments

As shown in Table 1, ligation of one uterine horn early in pregnancy reduced by one-half the number of placentomes that attached to the trophoblast but did not affect significantly the weight of the intact placentae, the cotyledons or the fetuses. Measurements of the placenta of control twins

Table 1. Fetal and placental measurements from pregnant sheep

	Day of gestation			
	120 days		140 days	
	Control	Exp.	Control	Exp.
No. of ewes	5	4	10	12
Fetal wt (g)	1877 ± 56	$2044 \pm 25^*$	3649 ± 207	$3389 \pm 158^*$
Placental wt (g)	466 ± 31	$437 \pm 29^*$	479 ± 42	$378 \pm 29^*$
Cotyledon no.	82 ± 2	$43 \pm 7^\dagger$	78 ± 2	$42 \pm 4^\dagger$
Cotyledon wt (g)	260 ± 7	$285 \pm 18^*$	233 ± 23	$204 \pm 21^*$
Uterus wt (g)	353 ± 14	364 ± 22	663 ± 49	$472 \pm 33^\dagger$

Values are mean \pm s.e.m.

* $P > 0.05$ compared with control at each time.

† $P < 0.001$ compared with control at each time.

resembled those of singleton lambs from experimental ewes with respect to number and weight of cotyledons and weight of the intact placenta ($P > 0.05$).

Neither weight of placentae nor weight or number of cotyledons changed detectably between 120 and 140 days of gestation in control or experimental ewes (Table 1). As would be expected, fetal weight did increase, particularly in control ewes carrying twins ($P < 0.01$).

In each experimental ewe, the appearance of the two uterine horns differed markedly with a clear demarcation at the site of the ligature. Distal to the ligature, in the portion free of placenta, the myometrium was thinner, paler and more flaccid. The mucosa also was thinner and bled very little when cut and the caruncles were no more than 2–3 mm in diameter, less than one-tenth the size of those that had fused with the trophoblast in the opposite horn. Arteries and veins in the broad ligament and in the myometrium of the ligated horn had thinner walls and were smaller in diameter. In most instances (but more often at Day 140 than at Day 120), the lumen of the ligated horn was distended with thick, opaque fluid, which ranged in volume from a few millilitres to more than 1 litre. Results of analysis of this fluid have been reported (Bazer *et al.*, 1979). In 2 ewes, the membranes had slipped past the ligature and had formed attachments with caruncles in the ligated horn. These animals resembled control ewes in that the uterine horns developed symmetrically. Measurements of uterine blood flow and of hormones from these 2 ewes were not included in the analysis since these animals did not properly belong to either group.

Between Days 120 and 140 of gestation, mean fetal weight increased almost 2-fold (Table 1) and the range of fetal weights also increased (coefficients of variation were 7% and 17%, respectively). Ligation of a uterine horn did not affect the weight of singleton fetuses at 120 or 140 days gestation ($P > 0.05$).

Only 1 of 17 experimental ewes carried twins compared with 5 of 20 control ewes. Twins were smaller than singleton lambs regardless of whether the singletons were carried in one uterine horn or in two ($P < 0.01$). Twins carried by the experimental ewe were even smaller. Interaction between uterine ligation and twin pregnancy affected fetal weight significantly ($P < 0.01$ two-way ANOVA). The weights of the twins carried by the experimental ewe were 2485 and 1919 g. The placenta of the larger twin was primarily in the horn of the uterus while the placenta of the smaller fetus was mostly in the body, the short segment common to both horns being just above the cervix. The placenta of the larger twin was heavier (431 and 160 g) and had more cotyledons (42 and 23). The arteries and veins in the broad ligament of the pregnant horn in this ewe were particularly large, as were blood vessels that traversed the peritoneal surface of the vagina and cervix to reach the body of the uterus. Data from this animal were not included in Text-fig. 2.

At 120 days no statistically significant fetoplacental associations were detected in the control or experimental ewes ($P > 0.05$). Amongst control ewes with singletons, the weight of the uterus and the weight of the 140-day fetus were associated positively ($r = 0.89$; $P < 0.01$). This association was not detected in data from ligated ewes or from control ewes with twins ($r = 0.10$, $P > 0.05$).

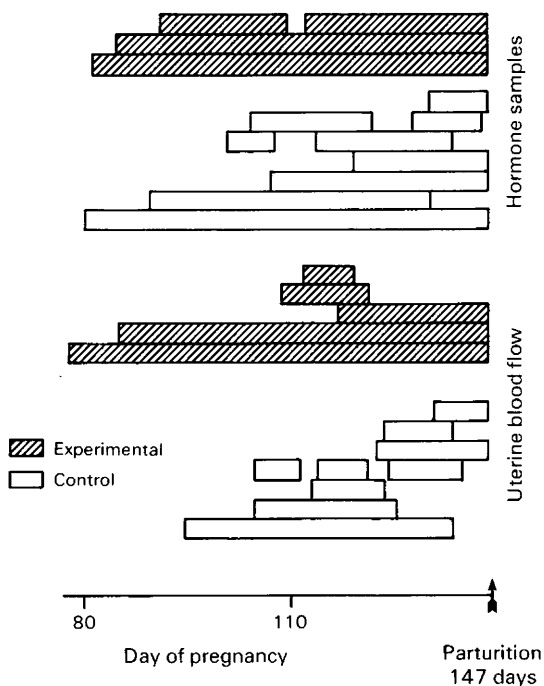
Generally, the weight of the fetus and the aggregate weight of the cotyledons of its placenta were associated positively; however, this is subject to several qualifications. First, the association was detected at 140 days, but not at 120 days, of gestation. In this period the weight of the average fetus nearly doubled whereas the weight of cotyledons tended to decrease. Second, the association was detected only when the uterine endometrial surface was limited, i.e. only in experimental ewes and control ewes with twins. For example, when data from these two groups were combined, correlation coefficients between weight of lambs at birth and weight and number of cotyledons were $r = 0.578$ ($P < 0.01$) and $r = 0.624$ ($P < 0.01$), respectively. In contrast, correlation coefficients between these same measurements from control ewes with singletons were $r = 0.256$ ($P > 0.05$) and $r = 0.171$ ($P > 0.05$), respectively.

Chronic experiments

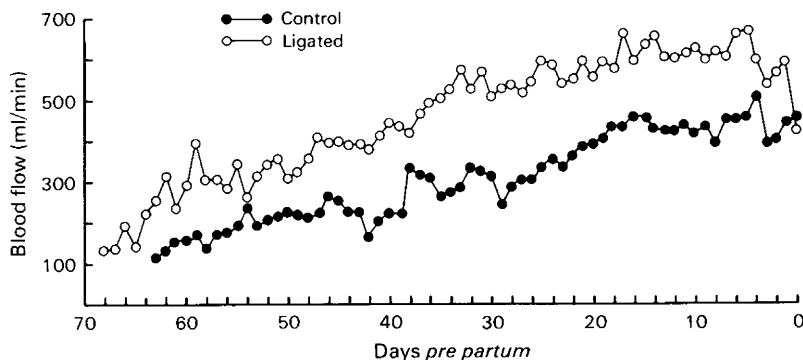
Animals were prepared on different days of pregnancy. Flow transducers or, more often,

catheters sometimes failed before the animals delivered at term and so estimates for hormone concentrations and uterine blood flow are not available for every animal every day. In one experimental ewe the membranes slipped past the ligature; data from this animal were not included. The numbers and distribution of each measurement for ewes carrying single fetuses are given in Text-fig. 1.

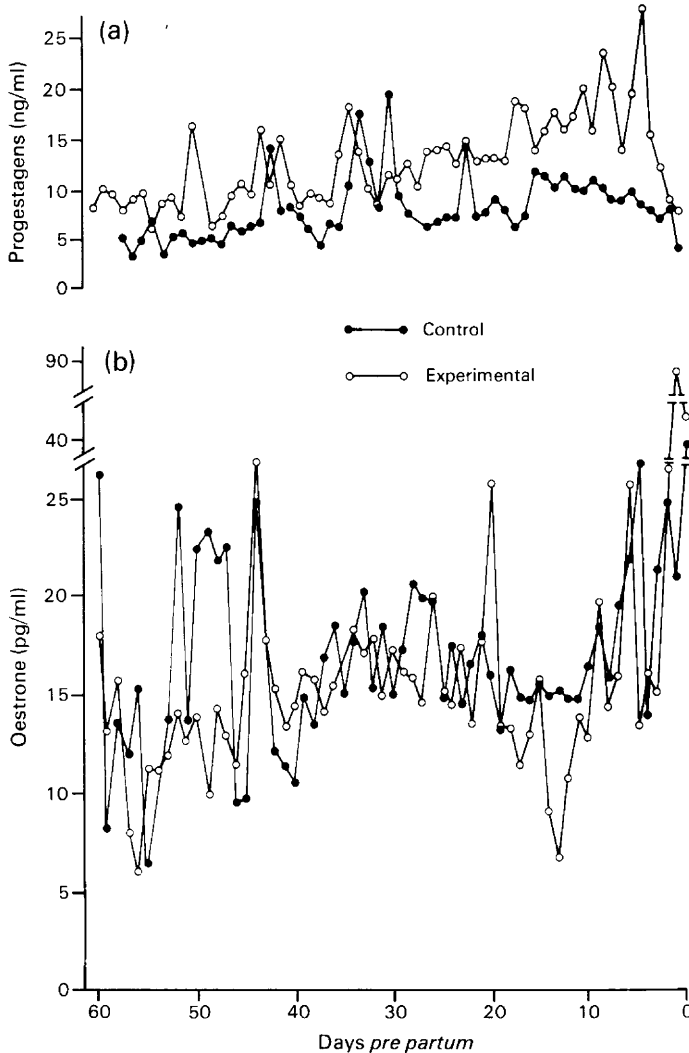
The rate of uterine blood flow to the pregnant horn of experimental ewes was higher than that to the horn ipsilateral to the corpus luteum of control animals; the difference was apparent as early as the 90th day of pregnancy (60 days *pre partum*) (Text-fig. 2). Response curves for data from the two



Text-fig. 1. Distribution of measurements of uterine blood flow and concentrations of arterial hormones of control and experimental ewes. Each bar represents data from a different ewe.



Text-fig. 2. Least square means of uterine blood flow of control and experimental ewes by day of pregnancy. Measurements from control ewes were from the uterine horn ipsilateral to the ovary with the corpus luteum; flow in the experimental animals was measured from the artery serving the uterine horn that contained the fetus.



Text-fig. 3. Least square means of arterial concentrations of (a) progestagens and (b) oestrone of control and experimental animals by day of pregnancy.

groups differ according to a test for heterogeneity of regression ($P < 0.01$). Progesteragen concentrations in arterial blood were higher in experimental animals ($P < 0.01$) (Text-fig. 3a), but there were no differences between groups for oestrone (Text-fig. 3b) or oestradiol (data not shown) ($P > 0.05$ for both).

Discussion

Ligation of a uterine horn early in pregnancy reduced the endometrial surface available for placental development but had no measurable effect on the weight of singletons at 120 or 140 days gestation. This agrees with earlier studies (Emmanouilides, Townsend & Bauer, 1968; Cefalo, Simkovich, Abel, Hellegers & Chez, 1977; Robinson, Kingston, Jones & Thorburn, 1979; Novy, Auvert, Kaplan & Grumbach, 1981; Clapp, Szeto, Larrow, Hewitt & Mann, 1981) in which there

also was evidence of a compensatory response; either the fetus achieved normal birth weight or fetal blood gases returned toward normal after the initial disturbance, if it were not too severe.

The present work suggests that overgrowth of functional placentomes and a greater than normal rate of blood flow to the horn containing the fetus are two components of a compensatory response. This too is in accord with other work (Metcalf *et al.*, 1962; Makowski *et al.*, 1968) at high altitude in which a higher rate of uterine blood flow and differences in placental gas exchange were observed, which suggested an increased diffusion capacity of the placenta. The circumstances of this study leave open the nature of the adaptation, whether it was genetically characteristic of the flock or an individual response during pregnancy. The present work supports the latter possibility.

In neither the experimental nor the control groups was there a detectable relationship between fetal weight and cotyledon weight or number at 120 days, but at 140 days there was, albeit only amongst those ewes in which there was a restriction of endometrial surface area, either by ligation or by twin pregnancy. This poses interesting questions about the developmental relationships between fetal growth and cotyledon weight and number. Is it possible that cotyledons achieve a certain weight regardless of number?

In view of the relationships mentioned above, factors that limit fetal size in twin pregnancy can be considered. No less space was available to twins than to singletons carried by ewes with a ligated horn. These two groups did not differ with respect to number or weight of cotyledons ($P > 0.05$). Yet twins were smaller ($P < 0.01$), which suggests that the factor limiting the fetal growth of twins is not simply the area of the uterus lumen available.

The significance of the increased concentrations of progestagens amongst experimental ewes also merits consideration. Given in pharmacological doses, this hormone will induce overgrowth of caruncles (Alexander & Williams, 1966) and growth of uterine blood vessels (Phelps, 1946; Williams, 1948; Caton *et al.*, 1974a) and does affect regulation of uterine blood flow (Caton *et al.*, 1974a; Caton, Abrams, Lackore, James & Barron, 1974b; Caton *et al.*, 1980; Roman-Ponce, Caton, Thatcher & Lehrer, 1983). Ewes differ with respect to the amount of progestagen released per kg of fetal, placental and uterine tissues during the last weeks of pregnancy and this amount is proportional to the subsequent weight of the lamb (Caton, Kalra & Wilcox, 1983). Moreover, maternal concentrations of progestagens are higher when ewes carry twins and are higher yet when they carry triplets (Emady, Hadley, Noakes & Arthur, 1974). In short, progestagens can induce the type of response observed in the present experiments. In some circumstances, there are also measurable differences in progestagen metabolism amongst ewes that are related to fetal weight. In view of this, progestagens may be part of the compensatory response in the present experiments. Whether or not progestagens are responsible and whether they act topically (Chamberlain, Gardner & Allen, 1941; Hohn & Robson, 1950) or systemically can be tested experimentally.

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References

- Abraham, G.E., Swerdloff, R., Tulchinsky, D. & O'Dell, W.E. (1971) Radioimmunoassay of plasma progesterone. *J. clin. Endocr. Metab.* **34**, 241–245.
- Alexander, G. & Williams, D. (1966) Progesterone and placental development in the sheep. *J. Endocr.* **34**, 241–245.
- Barcroft, J. (1964) *Researches on Pre-natal Life*, p. 30. C. C. Thomas, Springfield.
- Bazer, F.W., Roberts, R.M., Basha, S.M.M., Zavy, M.T., Caton, D. & Barron, D.H. (1979) Method for obtaining ovine uterine secretions from unilaterally pregnant ewes. *J. Anim. Sci.* **49**, 1522–1527.
- Caton, D., Abrams, R.M., Clapp, J.F. & Barron, D.H. (1974a) The effect of exogenous progesterone on the rate of blood flow of the uterus of ovariectomized sheep. *Q. Jl exp. Physiol.* **59**, 225–231.

- Caton, D., Abrams, R.M., Lackore, L.K., James, G. & Barron, D.H. (1974b) Effect of exogenous progesterone on the rates of uterine blood flow and O₂ consumption of sheep in early pregnancy. *Q. Jl exp. Physiol.* **59**, 233–239.
- Caton, D., Wilcox, C.J. & Kalra, P.S. (1980) Correlation of rate of uterine blood flow and plasma steroid concentrations at parturition in sheep. *J. Reprod. Fert.* **58**, 329–337.
- Caton, D., Kalra, P.S. & Wilcox, C.J. (1983) Relationships between endogenous maternal hormones during pregnancy and the weight of the lambs at birth. *Am. J. Physiol.* **244**, R31–R35.
- Cefalo, R.C., Simkovich, J.W., Abel, F., Hellegers, A.E. & Chez, R.A. (1977) Effect of potential placental surface area reduction on fetal growth. *Am. J. Obstet. Gynec.* **129**, 434–439.
- Chamberlain, T.L., Gardner, W.U. & Allen, E. (1941) Local responses of the sexual skin and mammary glands of monkeys to cutaneous applications of estrogen. *Endocrinology* **28**, 753–757.
- Clapp, J.F., Szeto, H.H., Larrow, R., Hewitt, J. & Mann, L.H. (1981) Fetal metabolic response to experimental placental vascular damage. *Am. J. Obstet. Gynec.* **140**, 446–451.
- Emady, M., Hadley, J.C., Noakes, D.E. & Arthur G.H. (1974) Progesterone level in the peripheral blood of pregnant ewes. *Vet. Rec.* **95**, 168–169.
- Emmanouilides, G.C., Townsend, D.O. & Bauer, R.A. (1968) Effects of single umbilical artery ligation in the lamb fetus. *Pediatrics* **42**, 919–927.
- Hohn, E.O. & Robson, J.M. (1950) The response of the rabbit's endometrium to uterine implants of progesterone and other steroids. *J. Physiol., Lond.* **111**, 174–183.
- Makowski, E.L., Battaglia, F.C., Meschia, G., Behrman, R.E., Schrufer, J., Seeds, A.E. & Bruns, P.O. (1968) Effect of maternal exposure to high altitude upon fetal oxygenation. *Am. J. Obstet. Gynec.* **100**, 852–861.
- Metcalfe, J., Meschia, G., Hellegers, A., Prystowsky, A., Huckabee, W. & Barron, D.H. (1962) Observations on the placental exchange of the respiratory gases of pregnant ewes at high altitude. *Q. Jl exp. Physiol.* **47**, 74, Abstr.
- Novy, M.J., Auvert, M.L., Kaplan, S.L. & Grumbach, M.M. (1981) Regulation of placental growth and chorionic somatomammotropin in the rhesus monkey: effects of protein deprivation, fetal anencephaly and placental vessel ligation. *Am. J. Obstet. Gynec.* **140**, 552–562.
- Phelps, D. (1946) Endometrial vascular reactions and the mechanism of nidation. *Am. J. Anat.* **79**, 167–180.
- Robinson, J.S., Kingston, E.J., Jones, C.T. & Thorburn, G.D. (1979) Studies on experimental growth retardation in sheep. The effect of removal of endometrial caruncles on fetal size and metabolism. *J. dev. Physiol.* **1**, 379–398.
- Roman-Ponce, H., Thatcher, W.W., Caton, D., Barron, D.H. & Wilcox, C.J. (1978) Effects of thermal stress and epinephrine on uterine blood flow in ewes. *J. Anim. Sci.* **46**, 167–174.
- Roman-Ponce, H., Caton, D., Thatcher, W.W. & Lehrer, R. (1983) Uterine blood flow in relation to endogenous hormones during the estrous cycle and early pregnancy. *Am. J. Physiol.* **245**, R843–R849.
- Williams, M.F. (1948) The vascular architecture of the rat uterus as influenced by estrogen and progesterone. *Am. J. Anat.* **83**, 247–308.

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