The progesterone content of rabbit uterine flushings

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Summary. A detailed study has been made of the progesterone content of uterine flushings taken from rabbits of different endocrine states. A high content of progesterone was recovered from the uterine lumen of pregnant and pseudopregnant rabbits. The amount of progesterone recovered in the flushings was not simply dependent on the concentration in the plasma, because plasma progesterone concentrations rose before detection of progesterone in the flushings and remained high after uterine progesterone content had fallen. Progesterone did not appear in the uterine fluid unless uterine protein was present. This was particularly evident in rabbits treated with progesterone in which, despite abnormally high plasma concentrations, little progesterone was found in uterine fluid until the protein content increased. After treatment of rabbits with oestrogen at the time of ovulation, uterine progesterone was present in higher amounts concomitant with an elevation of protein content although the peak values of progesterone and protein were delayed by 2–3 days. Plasma progesterone concentrations of about 5 ng/ml were necessary before progesterone appeared in the uterine lumen.

Introduction

The mammalian uterus may exert considerable control over the development of the embryo at the time of implantation. In all species examined, asynchrony between uterus and embryo leads to reduced rates of embryonic survival (Adams, 1973), and in many species an obligatory or facultative delay in implantation is evident (Wimsatt, 1975; Aitken, 1977). It is accepted that the steroid hormones acting via uterine receptors mediate this control process (O'Grady & Bell, 1977; Surani, 1977). Attention has concentrated on elucidating the linking mechanisms whereby the effects of the steroid-receptor interaction are transmitted to the embryo. The possibility also exists, however, of a direct effect of steroids on the embryo.

Preliminary reports have indicated that progesterone may be present in the fluid of the uterine lumen in very high concentrations during early pregnancy (Fowler, Johnson, Walters & Pratt, 1976; Cowan, Manes & Hagerman, 1976). It is possible therefore that a direct effect of steroids on the preimplantation embryo may be a component of the maternal control of embryonic development in some species. Steroids could operate either directly on embryo physiology or be used as a substrate for conversion to other steroids. The preimplantation pig embryo can effect conversion of progesterone to oestrogens (Gadsby, Burton, Heap & Perry, 1976), and Dickmann, Dey & Gupta (1975) claim to have detected histochemically some of the enzymes involved in steroid interconversion in a number of other species. It is therefore possible that any oestrogens produced by the embryo from maternal substrates could serve to convey information to oestrogen-binding sites in the corpus luteum (Perry, Heap, Burton & Gadsby, 1976), the uterus, or within the embryo itself (Bullock & Bhatt, 1973). We report here the results of a detailed study of steroid content of uterine flushings taken from rabbits in different endocrine states.

Materials and Methods

Animals

The New Zealand White rabbits were virgins (6–9 months old) and were divided into 5 groups. Those in Group 1 were mated twice to intact bucks; females in Group 2 were mated twice to vasectomized bucks; 100 i.u. HCG were injected subcutaneously to those in Group 3; does in Group 4 were

given daily intramuscular injections of 3 mg progesterone/kg in arachis oil; and does in Group 5 were given 100 i.u. HCG s.c. followed by two i.m. injections (100 µg each) of oestradiol benzoate in arachis oil at 6 and 30 h later (Adams, 1973). For each group, Day 0 was taken to be 12 h after the injection of HCG or mating.

Collection of samples

Animals were anaesthetized with sodium pentobarbital (Nembutal: Abbott). Blood samples were withdrawn by cardiac puncture into heparinized syringes, and plasma samples were prepared by centrifugation at 4°C and stored at -40°C. Uterine horns were exposed, clamped at the vagino-cervical junction, and 2 ml cold sterile 0.9% NaCl were injected into each horn at the uterotubal junction and then gently withdrawn. For Group 5, 5 ml NaCl were used for each horn. Samples from each horn of a doe were pooled, centrifuged at 14,000 g for 30 min at 4°C and stored at -40°C. Corpora lutea in each ovary were counted.

Protein estimations

The total protein content of the uterine flushings was estimated by the technique of Lowry, Rosebrough, Farr & Randall (1951): samples of flushings were applied to a G100 Sephadex column, and the eluted fractions were scanned for protein content. The third peak, which has previously been shown to be 90% or more uteroglobin (Johnson, 1972), was used to provide an estimate of uteroglobin content.

Steroid estimations

Steroids in uterine flushings and matching plasma samples were measured by radioimmunoassays (Abraham, Hopper, Tulchinsky, Swerdloff & Odell, 1971; Abraham, 1974; Abraham, Manlimos, Solis, Garza & Maroulis, 1975a; Abraham, Manlimos, Solis & Wickman, 1975b). Progesterone was assayed in all samples and oestradiol-17β, oestrone and androstenedione in samples from Groups 1 and 4.

Steroids in plasma and uterine fluids were extracted with ether after addition of the appropriate tritiated steroid containing approximately 2000 d/min radioactivity as the internal standard for recovery estimates. Steroids in the ether extract were separated on celite microcolumns by the procedure described by Abraham, Buster, Lucas, Corrales & Toller (1972), with a stationary phase of ethylene glycol. Three fractions were eluted, the first with 3.5 ml iso-octane, the second with 3.5 ml 10% ethyl acetate in iso-octane and the third with 3.5 ml 40% ethyl acetate in iso-octane.

All the antisera used in the assays were kindly supplied by Dr G. E. Abraham. After incubating the purified steroid fraction with the specific antiserum overnight at 4°C, the separation of bound from free steroid was effected by the addition of Dextran-coated charcoal. Triplicate measurements were used to establish the standard curve for each assay and duplicate samples were used to estimate the steroid concentration in uterine flushings and plasma samples. The concentration was calculated by interpolation from the standard curve which took the form of a non-linear asymptotic model which had been fitted to the standard curve by iterative methods (Walters, 1974). The mean of three blank samples was subtracted from the steroid levels in the sample before correction for recovery, the blanks being < 10 pg in all assays.

The specificity and sensitivity of the antisera used in the present work and the accuracy of the method has been extensively tested by Abraham and his colleagues and was confirmed in the present study. In experiments designed to test the accuracy of the method by adding known quantities of progesterone to 1 ml water samples, the value of measured progesterone did not differ by more than 10–15% from the expected value. Since only 30% of the total steroid present in each sample was used in the assay procedure and recovery estimates were between 60 and 80%, the smallest amount of steroid that could be measured per sample was 0.04 ng for progesterone, oestrone and androstenedione and 0.03 ng for oestradiol-17β. The precision of the assay was evaluated by duplicate measure-

ments of the assay sample in the same or different assays. The average coefficient of variation ranged from 10.2 to 11.8% within assays and from 11.4 to 14.7% between assays.

Results

Statistical examination of the temporal pattern of protein and progesterone content of uterine flushings suggested that there were no differences between the pregnant and pseudopregnant animals (Tables 1–3). The data for Groups 1, 2 and 3 were therefore combined for presentation in Text-fig. 1.

Table 1. Concentration (range in parentheses) of progesterone in plasma, and total quantities of progesterone and protein in the uterine flushings of pregnant rabbits (Group 1)

Day of pregnancy	No. of animals	Total protein (mg/uterus)	Total progesterone (ng/uterus)	Plasma progesterone conc. (ng/ml)
1½	3	0.08 (0.01–0.32)	0	0.27 (0.24–0.30)
2½	3	0.51 (0.10–0.92)	0	1.61 (1.55–1.65)
3½	3	1.48 (0.54–2.35)	1.24 (0.56–2.08)	3.06 (2.48–4.11)
4½	3	4.03 (2.98–5.25)	5.98 (4.74–7.23)	4.90 (4.14–5.89)
5½	3	2.90 (2.45–3.68)	20.49 (12.24–31.49)	5.88 (3.49–9.25)
6½	3	1.14 (1.10–1.20)	21.96 (14.66–33.47)	4.67 (4.21–5.24)
7½	3	3.82 (1.34–5.13)	11.50 (8.86–15.26)	7.31 (6.21–8.53)

Table 2. Levels (range in parentheses) of total protein and progesterone in uterine flushings, and plasma progesterone concentrations, of pseudopregnant rabbits (Group 2)

Day of pregnancy	No. of animals	Total protein (mg/uterus)	Total progesterone (ng/uterus)	Plasma progesterone conc. (ng/ml)
½	3	0.27 (0.20–0.38)	0	0.20 (0.3–2.6)
1½	3	0.12 (0.01–0.24)	0	0.73 (0.08–0.14)
2½	3	0.66 (0.21–0.96)	0.51 (0.1–1.52)	1.71 (1.40–2.28)
3½	3	2.39 (1.46–3.56)	1.06 (0.46–1.46)	3.05 (1.41–4.38)
4½	3	2.41 (1.21–3.56)	0.78 (0.29–1.55)	3.05 (1.60–4.13)
5½	3	2.87 (0.59–4.02)	36.52 (27.34–41.71)	7.28 (4.87–6.89)
6½	3	2.53 (1.20–3.40)	45.32 (38.64–49.86)	7.54 (5.23–11.54)
7½	3	2.19 (1.82–2.73)	39.28 (29.93–45.32)	8.06 (5.84–11.40)
8½	3	0.77 (0.51–1.14)	9.25 (4.87–12.29)	5.22 (4.60–6.37)
9½	3	0.64 (0.40–1.00)	7.80 (1.33–15.53)	6.36 (4.18–10.16)
10½	3	0.58 (0.47–0.71)	1.60 (0.40–3.82)	6.03 (3.24–11.32)
11½	3	0.64 (0.50–0.78)	1.61 (0.3–9.3)	5.11 (3.33–7.31)

Table 3. Levels (range in parentheses) of total protein and progesterone in uterine flushings, and of plasma progesterone concentration, of pseudopregnant rabbits (Group 3)

Day of pseudo-pregnancy	No. of animals	Total protein (mg/uterus)	Total progesterone (ng/uterus)	Plasma progesterone conc. (ng/ml)
½	3	0.40 (0.08–1.00)	0.18 (0.0–0.28)	0.25 (0.13–0.33)
1½	3	0.54 (0.38–0.73)	0.12 (0.0–0.35)	0.77 (0.60–0.96)
2½	3	1.05 (0.76–1.38)	0.06 (0.0–0.18)	0.97 (0.73–2.00)
3½	3	3.38 (2.54–5.30)	1.44 (0.98–1.89)	4.58 (3.78–5.38)
4½	3	3.91 (3.40–4.40)	11.48 (5.48–20.45)	5.50 (2.10–10.11)
5½	3	1.96 (1.60–2.65)	17.44 (14.23–19.82)	4.91 (4.19–5.74)
6½	3	2.74 (2.35–3.32)	31.79 (22.88–46.30)	4.28 (3.45–4.87)
7½	3	1.91 (1.20–2.76)	19.55 (0.77–30.80)	7.71 (5.55–9.97)
8½	3	1.15 (0.78–1.38)	24.33 (13.90–43.25)	11.71 (8.11–16.71)
9½	3	1.24 (0.19–3.14)	13.99 (1.47–37.94)	4.85 (4.01–5.69)
10½	3	0.50 (0.20–0.90)	3.47 (0.65–8.54)	3.98 (3.23–5.18)
11½	3	0.32 (0.13–0.43)	1.06 (0.97–1.31)	11.81 (1.88–26.02)

Text-fig. 1. The mean concentrations (± 1 S.E.) of progesterone and protein in uterine flushings and corresponding progesterone concentrations in the plasma of pregnant and pseudopregnant rabbits.

The plasma progesterone concentration rose sharply at 3 days to reach a fairly high level (> 5 ng/ml) by 4–5 days which was then maintained throughout the experimental period. The content of protein in uterine flushings, and in particular of uteroglobin (Table 4), showed a substantial increase at Day 3, whereas the increase in the content of uterine progesterone was not observed until 4½ days. No samples were collected at 7½ days in pregnant animals because flushing after this time damaged the implanting embryos and caused increased amounts of uterine protein. The steady decline in content of uterine progesterone and protein in pseudopregnant animals from 8½ days onwards was not matched by a similar decline in the plasma concentration of progesterone.

In the Group 4 rabbits, plasma progesterone concentrations at all times greatly exceeded those encountered in normal pregnancy and pseudopregnancy, but the content of uterine progesterone remained low until uterine protein content was elevated (Table 5). The correlation coefficient between

uterine progesterone content and plasma progesterone concentration was 0.31 (not significant) whereas the correlation between the uterine content of progesterone and that of protein was highly significant ($r = 0.80$, $P < 0.001$). All correlation coefficients were calculated on logarithmically transformed variables.

Injection of high levels of oestradiol benzoate at 6 and 30 h after LH administration has been reported to change the temporal profile of uterine secretions (Beier, Kühnel & Petri, 1971) and the synchronization requirements of the uterus (Beier, Mootz & Kühnel, 1972; Adams, 1973). Compared with those of pseudopregnant and pregnant animals (Groups 1, 2 and 3) plasma progesterone

Table 4. Levels (range in parentheses) of total uteroglobin recovered in uterine flushings of pseudopregnant rabbits (Group 2)

Day of pseudopregnancy	No. of animals	Total uteroglobin (mg/uterus)	Uteroglobin (as % of total protein)
$\frac{1}{2}$	2	0	0
$1\frac{1}{2}$	2	0	0
$2\frac{1}{2}$	2	0	0
$3\frac{1}{2}$	2	1.15 (0.99–1.32)	48.12
$4\frac{1}{2}$	2	1.72 (1.45–1.99)	71.37
$5\frac{1}{2}$	2	1.72 (0.47–2.98)	59.93
$6\frac{1}{2}$	2	1.72 (1.50–1.94)	67.98
$7\frac{1}{2}$	2	0.95 (0.90–1.50)	43.48
$8\frac{1}{2}$	2	0.42 (0.32–0.52)	54.55
$9\frac{1}{2}$	2	0.35 (0.30–0.39)	54.69
$10\frac{1}{2}$	2	0.15 (0.14–0.15)	25.86
$11\frac{1}{2}$	2	0.37 (0.09–0.28)	57.81

Table 5. Levels (range in parentheses) of total protein and progesterone in uterine flushings, and of plasma progesterone concentration, of rabbits injected daily with 3 mg progesterone/kg (Group 4)

Days after initial progesterone injection	No. of animals	Total protein (mg/uterus)	Total progesterone (ng/uterus)	Plasma progesterone conc. (ng/ml)
1	3	0.02 (0–0.03)	0.24 (0–0.34)	15.58 (15.38–15.82)
2	3	0.15 (0.02–0.24)	0.89 (0.20–1.51)	28.72 (16.97–48.40)
3	3	0.94 (0.72–1.07)	3.92 (2.59–5.87)	35.93 (32.48–39.90)
4	3	1.03 (0.68–1.22)	10.26 (5.32–13.93)	30.50 (22.29–29.56)
5	3	1.26 (1.10–1.45)	6.92 (3.35–11.37)	14.25 (10.59–17.16)

Table 6. Levels (range in parentheses) of total protein and progesterone in uterine flushings, and of plasma progesterone concentration, of rabbits injected with HCG, and with 100 µg oestrogen at 6 h and 30 h later

Day of pseudo-pregnancy	No. of animals	Total protein (mg/uterus)	Total progesterone (ng/uterus)	Plasma progesterone conc. (ng/ml)
$1\frac{1}{2}$	1	0	6.11	2.12
$2\frac{1}{2}$	1	1.25	14.84	10.30
$3\frac{1}{2}$	1	1.15	1.99	9.73
$4\frac{1}{2}$	2	1.84 (0.15–3.53)	4.93 (0.30–9.55)	7.15 (2.60–11.70)
$5\frac{1}{2}$	1	2.54	8.48	9.27
$6\frac{1}{2}$	2	6.14 (4.50–7.88)	6.62 (2.04–11.20)	13.00 (12.42–13.58)
$7\frac{1}{2}$	2	7.25 (6.98–7.53)	20.94 (9.54–32.35)	10.36 (9.65–11.08)
$8\frac{1}{2}$	3	5.82 (5.73–5.95)	61.23 (19.91–83.00)	15.29 (4.49–24.71)
$9\frac{1}{2}$	2	4.48 (3.53–5.43)	73.79 (26.13–121.44)	31.72 (17.38–46.07)
$10\frac{1}{2}$	2	3.80 (1.57–6.02)	85.69 (33.11–138.27)	14.55 (14.33–14.75)
$11\frac{1}{2}$	1	2.0	26.42	10.09
$12\frac{1}{2}$	2	0.15 (0.14–0.16)	6.24 (4.81–7.67)	8.03 (7.09–8.96)

concentrations in Group 5 rabbits rose earlier and to higher levels, uterine protein content rose at the same time but reached a later and higher peak, and the initial small rise in amounts of uterine progesterone was followed by a delayed rise to higher levels concomitant with that of the uterine protein in these flushings (Table 6).

The concentrations of some other steroids were measured in Groups 1 and 4. Plasma oestradiol-17 β values between 1½ and 7½ days of gestation were between 10 and 70 pg/ml, and similar to published values (Challis, Davies & Ryan, 1973), but no oestradiol-17 β or oestrone was found in the uterine flushings of either group. The levels of plasma androstenedione in rabbits treated with exogenous progesterone (Group 4: 0.52 ng/ml) were elevated compared with those of pregnant rabbits (Group 1: 0.03 ng/ml), but the levels of androstenedione in uterine flushings were negligible in both groups. The high levels of plasma androstenedione in Group 4 animals probably resulted from the peripheral conversion of progesterone to androstenedione. Because levels of oestradiol-17 β , oestrone or androstenedione were evidently negligible in uterine flushings, no further analyses of these steroids was undertaken in Groups 2, 3 or 5.

Discussion

These results demonstrate that large amounts of progesterone may be recovered from the uterine lumen of the progestational rabbit. Assuming a uterine fluid volume of 0.25 ml per horn, the mean peak concentration of progesterone in uterine fluid of pregnant and pseudopregnant rabbits ranged between 44 and 90 ng/ml. Since recovery was undoubtedly not 100% and since uterine fluid volume may be less than 0.25 ml, this calculated concentration of progesterone is likely to be a substantial underestimate. This figure is only slightly lower than our preliminary estimate (138 ng/ml) based on a small series of pseudopregnant rabbits in which the response to HCG was very variable (Fowler *et al.*, 1976). Only trace amounts of the other steroids assayed were detected in uterine flushings despite appreciable plasma levels of one of these, androstenedione. The amount of progesterone recovered in the flushings did not follow the same pattern as plasma progesterone concentrations; for example, in pregnant and pseudopregnant animals (Groups 1–3) the plasma progesterone concentration rose before the progesterone content of the flushings, and remained high after the uterine content had fallen. The dissociation of uterine and plasma progesterone was particularly marked in the animals given daily progesterone injections (Group 4), in which plasma levels were much higher than those found in pseudopregnancy or pregnancy but uterine levels were lower. The passage of progesterone into the uterine lumen is not, therefore, simply dependent upon the level present in the plasma.

We have previously reported that a minimum of 80% of progesterone recovered in uterine flushings is bound to protein (Fowler *et al.*, 1976) and that the amount of recovered progesterone and protein in uterine flushings were positively correlated. In the more extensive data presented here, it is clear that progesterone does not appear in the uterine fluid unless uterine protein is also present. Not only is this shown in the pregnant and pseudopregnant animals, but it is also particularly evident in progesterone-treated animals (Group 4) in which, despite the abnormally high plasma concentrations, little progesterone was found in uterine fluid until the content of uterine protein increased. In addition, in animals given oestrogen at the time of ovulation (Group 5), the peak values for the progesterone and protein content in the flushings were delayed by 2–3 days and both were considerably elevated compared with the values in untreated pseudopregnant rabbits (Group 2). It therefore seems reasonable to conclude that as long as progesterone levels in the plasma are adequate (around 5 ng/ml), the appearance of the steroid in the uterine lumen is in large part regulated by the presence there of progesterone-binding proteins. The only protein from rabbit uterine flushings that has been shown to give quantitatively significant progesterone binding *in vitro* is uteroglobin (Beato & Baier, 1975), and it seems probable that the uteroglobin also binds the progesterone *in vivo*.

It has been reported that low levels of uteroglobin and its mRNA are synthesized and secreted under the influence of oestrogens alone, higher levels are synthesized after progesterone injection alone, but maximal synthesis is achieved by a sequential oestrogen and progesterone stimulation (Beato & Arneman, 1975; Rahman, Billiar & Little, 1975; Beato & Nieto, 1976; Beato, 1977). In our study, uterine protein levels were also lower with progesterone alone (Group 4), and despite the

maintained presence of progesterone in pseudopregnant animals (Groups 2 and 3), uterine protein and uteroglobin levels fell after several days. In contrast, with oestrogen prestimulation (Group 5), the highest amounts of uterine protein were achieved. The oestrogen may act directly on the uterus to stimulate at the transcriptional or translational level, but the delay in occurrence of the peak of uterine protein after oestrogen stimulation might argue against this hypothesis. Alternatively, or in addition, oestrogen might act indirectly by stimulating the secretory activity of the the corpora lutea (Johnson & Murray, 1974). The high plasma progesterone concentrations found in Group 5 would support such an action.

It is necessary to explain why, at plasma progesterone levels lower than 5 ng/ml, uterine progesterone content is generally low even in the presence of increased protein. This situation may arise because the affinity of the reduced form of uteroglobin for progesterone has been reported to be only 3×10^{-6} (Beato, 1976), which is lower than the affinities of progesterone for intracellular endometrial receptors or serum protein carriers (McGuire & Bariso, 1972; Milgrom, Allouch, Alger & Baulieu, 1973). Another explanation might be that some intermediary transport mechanism, which is dependent on higher progesterone levels than those stimulating uteroglobin secretion, must be activated before progesterone appears in the uterine lumen. It may also be that 20α -dihydroprogesterone competes with progesterone for binding to protein in early pregnancy. The 20α -dihydroprogesterone concentration is similar to that of progesterone in plasma from pregnant rabbits and even higher in pseudopregnant rabbits (Fuchs & Beling, 1974). However, since Beato (1976) has shown that the relative affinity of 20α -dihydroprogesterone for uteroglobin is low compared with progesterone this explanation appears unlikely. We are currently investigating these different possibilities.

The functional significance of progesterone in the uterine lumen is not established. Progesterone in high concentrations directly affects the stability of cell membranes (Weissmann, 1972) and may be immunosuppressive (see Carter, 1976). However, the progesterone concentrations estimated from data presented in this study (44–90 ng/ml uterine fluid) are well below those known to exert an embryotoxic effect (Daniel & Levy, 1964). The uteroglobin–progesterone complex may exert an endocrine effect on embryonic development or the interaction of the embryo with the uterus. It is possible that uteroglobin acts as a carrier of progesterone from maternal tissue to the embryo. Two reports have suggested that progesterone in the presence of binding proteins stimulates cell division and blastocyst expansion (El-Banna & Daniel, 1972; Roblero & Izquierdo, 1976), and uteroglobin has been shown to penetrate rabbit blastocysts (Kirchner, 1972). Pig blastocysts can utilize progesterone as a substrate for oestrogen synthesis (Gadsby *et al.*, 1976) and 6-day rabbit blastocysts can synthesize cholesterol and pregnenolone from acetate and undertake certain steroid conversions (Huff & Eik-Ness, 1966). These observations raise the possibility at least of a steroidal influence by the embryos of some species on uterine receptivity.

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References

- ABRAHAM, G.E. (1974) Radioimmunoassay of steroids in biological material. *Acta endocr., Copenh., Suppl.* **183**, 7–42.
- ABRAHAM, G.E., BUSTER, J.E., LUCAS, L.A., CORRALES, P.C. & TOLLER, R.C. (1972) Chromatographic separation of steroid hormones for use in radioimmunoassay. *Analyt. Letters* **5**, 509–517.
- ABRAHAM, G.E., HOPPER, K., TULCHINSKY, D., SWERDLOFF, R.S. & ODELL, W.D. (1971) Simultaneous measurement of plasma progesterone, 17 -hydroxyprogesterone and oestradiol- 17β by radioimmunoassay. *Analyt. Letters* **4**, 325–335.
- ABRAHAM, G.E., MANLIMOS, F.S., SOLIS, M., GARZA, R. & MAROULIS, G.B. (1975a) Combined radioimmunoassay of four steroids in one ml of plasma. I. Progestins. *Clin. Biochem.* **8**, 369–373.
- ABRAHAM, G.E., MANLIMOS, F.S., SOLIS, M. & WICKMAN, A.C. (1975b) Combined radioimmunoassay of four steroids in one ml of plasma. II. Androgens. *Clin. Biochem.* **8**, 374–378.

- ADAMS, C.E. (1973) Asynchronous egg transfer in the rabbit. *J. Reprod. Fert.* **35**, 613–614.
- AITKEN, R.J. (1977) Embryonic diapause. In *Development in Mammals*, Vol. 1, pp. 307–360. Ed. M. H. Johnson. Elsevier North-Holland Biomedical Press, Amsterdam.
- BEATO, M. (1976) Binding of steroids to uteroglobin. *J. Steroid Biochem.* **7**, 327–334.
- BEATO, M. (1977) Physico-chemical characterization of uteroglobin and its interaction with progesterone. In *Development in Mammals*, Vol. 2, pp. 173–198. Ed. M. H. Johnson. Elsevier North-Holland Biomedical Press, Amsterdam (in press).
- BEATO, M. & ARNEMAN, J. (1975) Hormone-dependent synthesis and secretion of uteroglobin in isolated rabbit uterus. *FEBS Letters* **58**, 126–129.
- BEATO, M. & BAIER, R. (1975) Binding of progesterone to the proteins of the uterine luminal fluid. Identification of uteroglobin as the binding protein. *Biochim. biophys. Acta* **392**, 346–356.
- BEATO, M. & NIETO, A. (1976) Translation of the mRNA for rabbit uteroglobin in cell-free systems. *Eur. J. Biochem.* **64**, 15–25.
- BEIER, H.M., KÜHNEL, W. & PETRI, G. (1971) Uterine secretion proteins as extrinsic factors in preimplantation development. *Adv. Biosci.* **6**, 165–190.
- BEIER, H.M., MOOTZ, U. & KÜHNEL, W. (1972) Asynchrone Eitransplantationen während der verzögerten Uterussekrektion beim Kaninchen. In *Proc. 7th Int. Congr. Anim. Reprod. & A. I., Munich*, Vol. 3, p. 1891.
- BULLOCK, D.W. & BHATT, B.M. (1973) Oestrogen binding to rabbit blastocysts and its possible role in implantation. *J. Reprod. Fert.* **35**, 614–615.
- CARTER, J. (1976) The effect of progesterone, oestradiol and HCG on cell-mediated immunity in pregnant mice. *J. Reprod. Fert.* **46**, 211–216.
- CHALLIS, J.R.G., DAVIES, I.J. & RYAN, K.J. (1973) The concentrations of progesterone, estrone and estradiol-17 β in the plasma of pregnant rabbits. *Endocrinology* **93**, 971–976.
- COWAN, B.D., MANES, C. & HAGERMAN, D.D. (1976) Progesterone concentration in rabbit uterine flushings before implantation. *J. Reprod. Fert.* **47**, 359–361.
- DANIEL, J.C., JR & LEVY, J.D. (1964) The action of progesterone as a cleavage inhibitor of rabbit ova. *J. Reprod. Fert.* **7**, 323–333.
- DICKMAN, Z., DEY, S.K. & GUPTA, J.S. (1975) Steroidogenesis in rabbit preimplantation embryos. *Proc. natn. Acad. Sci. U.S.A.* **72**, 298–300.
- EL-BANNA, A.A. & DANIEL, J.C., JR (1972) Stimulation of rabbit blastocysts *in vitro* by progesterone and uterine proteins in combination. *Fert. Steril.* **23**, 102–104.
- FOWLER, R.E., JOHNSON, M.H., WALTERS, D.E. & PRATT, H.P.M. (1976) The progesterone and protein composition of rabbit uterine flushings. *J. Reprod. Fert.* **46**, 427–430.
- FUCHS, A.R. & BELING, C. (1974) Evidence for early ovarian recognition of blastocysts in rabbits. *Endocrinology* **95**, 1054–1058.
- GADSBY, J.E., BURTON, R.D., HEAP, R.B. & PERRY, J.S. (1976) Steroid metabolism and synthesis in early embryonic tissue of pig, sheep and cow. *J. Endocr.* **71**, 45–46P.
- HUFF, R.L. & EIK-NES, K.B. (1966) Metabolism *in vitro* of acetate and certain steroids by six-day-old rabbit blastocysts. *J. Reprod. Fert.* **11**, 57–63.
- JOHNSON, M.H. (1972) The protein composition of secretions from pregnant and pseudopregnant rabbit uteri with and without a copper intrauterine device. *Fert. Steril.* **23**, 123–130.
- JOHNSON, M.H. & MURRAY, F.A. (1974) Effect of dosage of oestradiol-17 β on the life-span of the rabbit corpus luteum. *Experientia* **30**, 308–309.
- KIRCHNER, C. (1972) Immune histologic studies on the synthesis of a uterine specific protein in the rabbit and its passage through the blastocyst coverings. *Fert. Steril.* **23**, 131–136.
- LOWRY, O.H., ROSEBROUGH, M.J., FARR, A.L. & RANDALL, R.J. (1951) Protein measurement with the Folin phenol reagent. *J. biol. Chem.* **193**, 265–275.
- MCGUIRE, J.L. & BARISO, C.D. (1972) Isolation and preliminary characterization of a progesterone specific binding macromolecule from the 273,000 g supernatant of rat and rabbit uteri. *Endocrinology* **90**, 496–506.
- MILGROM, K., ALLOUCH, P., ALGER, M. & BAULIEU, E.K. (1973) Progesterone-binding plasma protein of pregnant guinea pig. *J. biol. Chem.* **248**, 1106–1114.
- O'GRADY, J.E. & BELL, S.C. (1977) The role of the endometrium in blastocyst implantation. In *Development in Mammals*, Vol. 1, pp. 165–244. Ed. M. H. Johnson. Elsevier North-Holland Biomedical Press, Amsterdam.
- PERRY, J.S., HEAP, R.B., BURTON, R.D. & GADSBY, J.E. (1976) Endocrinology of the early embryo and its role in the establishment of pregnancy. *J. Reprod. Fert., Suppl.* **25**, 85–104.
- RAHMAN, S.S.U., BILLIAR, R.B. & LITTLE, B. (1975) Induction of uteroglobin in rabbits by progesterone, estradiol-17 β and ACTH. *Biol. Reprod.* **12**, 305–314.
- ROBLERO, L. & IZQUIERDO, L. (1976) Effect of progesterone on the cleavage rate of mouse embryos *in vitro*. *J. Reprod. Fert.* **46**, 475–476.
- SURANI, M.A.H. (1977) Cellular and molecular approaches to blastocyst uterine interactions at implantation. In *Development in Mammals*, Vol. 1, pp. 245–305. Ed. M. H. Johnson. Elsevier North-Holland Biomedical Press, Amsterdam.
- WALTERS, D.E. (1974) The use of asymptotic models in multiple hormone assays. *J. Roy. Stat. Soc. C* **23**, 43–50.
- WEISSMANN, G. (1972) The effect of steroids and drugs on lysosomes. In *Lysosomes in Biology and Pathology*, Vol. 1, pp. 276–295. Eds J. T. Dingle & H. B. Fell. North-Holland Publishing Co., Amsterdam.
- WIMSATT, W.A. (1975) Some comparative aspects of implantation. *Biol. Reprod.* **12**, 1–40.

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