Progesterone concentration in rabbit uterine flushings before implantation

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Uteroglobin (or blastokinin) is a specific protein produced by rabbit endometrium when stimulated by exogenous or endogenous progesterone (Schwick, 1965; Beier, 1968a, b; Krishnan & Daniel, 1967; Urzua, Stambaugh, Flickinger & Mastroianni, 1970; Arthur & Daniel, 1972; Bullock & Connell, 1973). The protein is known to bind steroids, particularly progesterone (Urzua *et al.*, 1970; Arthur & Daniel, 1972; Beato & Baier, 1975), and its role in early rabbit embryonic development may be that of a carrier protein for progesterone (Arthur, Cowan & Daniel, 1972; El Banna & Daniel, 1972a, b; Beato & Baier, 1975). Progesterone can be detected in 5- and 6-day *post coitum* rabbit blastocysts, 7-, 8- and 9-day *post coitum* blastocoelic fluid and in uterine washings from 5-day pseudopregnant rabbits (Beier, 1968b; Seamark & Lutwak-Mann, 1972). We now report measurements of the concentration of progesterone in non-pregnant and pregnant rabbit uterine flushings and plasma.

Methods

Mature virgin rabbits of the New Zealand breed were killed by cervical dislocation at oestrus and 1–6 days *post coitum*. The uteri were flushed with 5.0 ml 0.9% (w/v) sodium chloride solution through each horn from the oviducal end. The washings were centrifuged at 12,000 g at 3°C for 10 min, and the supernatant fluid was frozen at –20°C until analysed. An average of 7 embryos/animal was recovered. Blood samples were obtained by cardiac puncture with heparin as anticoagulant. The blood was centrifuged at 6000 g for 10 min, and the plasma was frozen. There were two animals in each group, and the individual samples were analysed in duplicate. Samples (0.5 ml) of plasma or uterine fluid were extracted with 5 ml petroleum ether. The extracts were dried under a stream of nitrogen at 22°C. Recoveries in the extraction process were measured by adding tritiated progesterone, 17 α -hydroxyprogesterone or corticosterone to replicate plasma or uterine fluid samples before extraction. The average recovery ($\pm$ S.D.) of progesterone was $84 \pm 5\%$, of 17 α -hydroxyprogesterone $47 \pm 6\%$, and of corticosterone $0.3 \pm 0.1\%$. The unknown amounts of progesterone in the uterine flushings were purified from petroleum ether extracts by chromatography through a Sephadex LH-20 (Pharmacia) column (Murphy, 1971; Carr, Mikhail & Flickinger, 1971). Recovery of progesterone from the column was greater than 90%. The specificity of the assay is such that the small amount of 17 α -hydroxyprogesterone present in plasma and the low affinity of 20 α -dihydroprogesterone for corticosterone-binding globulin (Johansson, 1969) made chromatography of the plasma samples before analysis unnecessary.

The competitive protein-binding assay (Murphy, Engelberg & Patlee, 1963; Neill, Johansson, Datta & Knobil, 1967) was employed for progesterone; dog serum was pretreated with Florisil to achieve a twofold increase of the binding of corticosterone under the assay conditions used.

The total protein of the uterine flushings was determined by the method of Lowry, Rosebrough, Farr & Randall (1951). Uteroglobin concentrations were determined by the method of Johnson, Cowan & Daniel (1972). The antibody was tested for specificity by a double immunodiffusion plate composed of 1% agar in saline. Samples (50 μ l) of antigen or antibody were placed in each well. The reaction of the antibody against uterine fluid of rabbits at 5 days *post coitum* is shown in Text-fig.

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1. The major protein fractions were obtained by Sephadex G-75 (Pharmacia) gel chromatography (Arthur *et al.*, 1972). The antibody was clearly suitable for use in the radial immunodiffusion analysis.

Text-fig. 1. A double immunodiffusion plate showing the presence of a single precipitin band to uterine flushings (UF) from a rabbit at 5 days *post coitum* and uteroglobin (Fraction III), and the absence of bands to Fractions I, II, and IV. The centre well contained a goat antiserum to uteroglobin and Fractions I, II, III and IV were obtained from chromatography of 5-day *post coitum* uterine fluid. The plate was photographed after 48 hr and no other bands had developed after 12 days.

The results of the progesterone-binding assay were calculated after log-logit transformation (Ekins & Newman, 1970). Linear regression analysis of the transformed data was applied to each standard curve, and the correlation coefficient was always greater than 0.989, demonstrating the precision of the method. Its specificity and accuracy have been described (Neill *et al.*, 1967), and the sensitivity of the assay (defined as the 95% confidence limit of the lowest (0.2 ng) point of the standard curve) was 0.1 ng.

Results

Regression analysis of the results (Table 1) showed that there was a statistically significant increase ($P < 0.01$) in the concentration of progesterone in plasma and uterine flushings. The latter was paralleled by an increase in uteroglobin and total protein concentration as expected. The amount of progesterone present in the uterine flushings probably cannot be accounted for by simple serum contamination because of the much higher progesterone:protein ratio in the flushings than in blood. Ultrafiltration of plasma as a source of the hormone cannot be ruled out, but specific secretion seems equally likely.

Table 1. The changes in the concentrations of progesterone, total protein and uteroglobin in the uterine flushings of rabbits at different times after mating

Days <i>post coitum</i>	Progesterone (ng/ml)		Protein (mg/ml)	Uteroglobin (mg/ml)
	Plasma	Uterine flushings	Uterine flushings	Uterine flushings
0	<0.2*	<0.2*	0.12	---*
1	<0.2*	<0.2*	0.20	---*
2	2.3, 1.3	<0.2*	0.18	---*
3	2.8, 2.5	<0.2*	0.20	0.04
4	5.2, 3.6	1.1, 0.8	0.50	0.18
5	5.4, 3.6	2.5, 1.4	0.90	0.28
6	6.9, 5.3	26.0, 18.4	1.14	0.36

* Below sensitivity of assay.

Pregnancy was associated therefore with increased progesterone, total protein and uteroglobin concentrations in uterine flushings 4–6 days after mating, the progesterone increase being the most rapid. By using a molecular weight of 15,000 for uteroglobin (Murray, McGaughy & Yarus, 1972), we calculate a ratio of one molecule of progesterone to 3000–4000 molecules of uteroglobin on Days 4 and 5 *post coitum*. On Day 6 there is about one molecule of progesterone to 400 molecules of uteroglobin. The reason for this abrupt change is unknown, and the precise role that progesterone in the rabbit uterine secretions plays in implantation and early embryonic development needs to be investigated further.

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