PROTEIN COMPOSITION OF RAT UTERINE FLUSHINGS

A Thesis Submitted for the Degree of

MASTER OF SCIENCE

by

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CONTENTS

Acknowledgements		III
Declaration		IV
Summary		V
1. Introduction		
	1.1 General	1
	1.2 The protein composition of the uterus in the various hormonal states of the oestrous cycle and on day 5 pseudopregnancy.	3
	1.3 Effect of unilateral IUCD on uterine protein compositions during the oestrous cycle and on day 5 pseudopregnancy.	9
2. Materials and Methods		
	2.1 Experimental animals	13
	2.2 Vaginal smears	13
	2.3 Surgical procedures	14
	2.4 Oestrogen-progesterone replacement therapy.	16
	2.5 Induction of Pseudopregnancy	16
	2.6 Flushing techniques	17
	2.7 Concentration procedures	17
	2.8 Effect of concentration on protein profiles.	17
	2.9 Analytical procedures.	18
	2.10 Experimental design	23

3. Results

3.1 An arbitary classification of Gel electrophoretic profiles. 30

3.2 The protein composition of the various hormonal states of the oestrous cycle and on day 5 pseudopregnancy. 31

3.3 Protein composition of the uterus of ovariectomized rats with and without oestrogen and progesterone replacement therapy. 32

3.4 Effect of unilateral IUCD on protein composition on day 5 psuedopregnancy. 33

3.5 Electrophoretic profile of rat plasma. 34

3.6 Comparison of the electrophoretic profiles of that of rat plasma and uterine flushings. 35

4. Discussion 55

5. Appendices 68

6. Bibliography 71

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DECLARATION

I declare that this thesis contains no material which has been accepted for the award of any other degree or diploma in any other university, and to the best of my knowledge contains no material previously published by any other persons, except where due reference is made in the text of the thesis.

The results of this thesis have also been presented to the meeting of the Australian Society for Reproductive Biology in Brisbane, August 1976.

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SUMMARY

A major protein component of uterine fluid has been described for the rabbit in early pregnancy and pseduo--pregnancy (Beier et al, 1971 and Daniel, 1971). Its function may be related to uptake of steroids and/or nutrients by the unimplanted embryo (Fowler et al, 1976). This component has not been described for rats, although secretory activity by uterine epithelial and glandular cells early in pregnancy has been claimed (Nilsson, 1972) but this has been challenged (Enders and Nelson, 1973 and Parr and Parr, 1974).

Using SDS-gel electrophoresis, originally developed by Maizel (1966) and modified by Schnaitman (1974), uterine flushings were obtained at either pro-oestrus, oestrus, dioestrus or day 5 pseudopregnancy and analysed after the protein level had been measured by Lowry assay (Lowry et al, 1951). Electrophoretic profiles of the gels were subsequently recorded both visually and with an optical densitometer.

At pro-oestrus (n=4), 7-16 bands occurred of which large peaks had Rf values of 0.5, 0.6, 0.9 (Post-albumin) and 1.0 (albumin). At oestrus (n=5), 11-14 bands occurred, Rf values of large peaks being the same as pro-oestrus. At dioestrus (n=5) and on day 5 pseduopregnancy (n=5) uterine flushings had lower total protein so these were pooled and concentrated. Although 11-17 and 12-14 bands occurred respectively, there were only two large peaks with Rf values of 0.9 and 1.0.

Preliminary study involving ovariectomized animals given replacement therapy of 1.0μg of oestradiol - 17β/day for 10 days either with (n=3) or without (n=3) 1 mg of progesterone/day for the last 5 days resulted in 11-15 and 10-12 protein bands respectively. In three out of six samples, large peaks had Rf values of 0.5, 0.6, 0.9 and 1.0. Thus these profiles are similar to those of pro-oestrus and oestrus. However, when 5 mg of progesterone/day was given for the last 5 days, the prominent protein peaks 5 and 6 were not apparent, whereas ovariectomy alone only resulted in albumin being visible.

Finally, this project was extended to include the study of the effect of IUCD on the protein composition of the uterus on day 5 pseudopregnancy (n=4). Protein levels in uterine flushings demonstrate that the IUCD significantly increased the protein levels ($P<0.05$) confirming previous reports (Kar et al, 1964 and Breed et al, 1972). However, the electrophoretic profiles of uterine flushings obtained from control and IUCD horns demonstrated 7-15 and 11-14 bands respectively with large peaks only having Rf values of 0.9 and 1.0 in both horns. Thus no qualitative differences in protein components between two horns were apparent even though quantitative differences were found.

In conclusion, therefore, when all protein peaks are considered, comparing uterine proteins with plasma, Rf values of 0.1, 0.2, 0.3, 0.4, 0.5, 0.9, 1.0, 1.2, and 3.0 were found in both plasma and uterine flusings irrespective of the endocrine states, but peaks with Rf values of 0.6, 0.7, 0.8 and 1.4 were present only in uterine flushings.

When different endocrine states are compared, uterine flushings obtained at pro-oestrus and oestrus had two components (Rf values 0.5 and 0.6) in greater amounts than in flushings taken at dioestrus and day 5 pseudopregnancy. By comparison with protein standards the molecular weights of these components are about 103,000 and 94,000 respectively. No extra protein bands were found in day 5 pseudopregnancy flushings. Thus it may be that oestrogen induces the increase of two proteins in uterine fluid, whereas it appears that progesterone does not induce any extra protein components on day 5 pseudopregnancy.