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OBSERVATIONS ON THE DISTRIBUTION OF UTERINE EGGS IN PSEUDOPREGNANT RABBITS

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The phenomenon of pseudopregnancy has been noticed in reproductive biology regarding implantation and pregnancy. Knowledge of the reproductive phenomena in pseudopregnant rabbits, however, still remains limited except for histology of the reproductive tract. Changes in the fine vascular structures of the endometrium attain a maximum at 6 to 7 days *post coitum* (*p. c.*) during pseudopregnancy in rabbit (TSUTSUMI and HAFEZ, 19). Cellular contents and state of mucus of the vaginal lumen in pseudopregnant does show rather similar variations seen in normal pregnant does, although there is a considerable variation among individual dose (TSUTSUMI and HACHINOHE, 21). Fluctuations of progesterin level in the ovary and ovarian venous blood were also investigated in pregnant and pseudopregnant does (8, 9, 12, 13, 15, 16). Patterns of secretion of progesterone and 20 α -hydroxypregn-4-en-3-one were different between pregnancy and pseudopregnancy, thus it is surmized that the maternal ovary recognizes the presence of blastocysts prior to implantation, and that the fertilized ova exerts a luteotropic influence already at the blastocyst stage.

Basically, physiological and morphological differences between pregnant and pseudopregnant does are due to eggs regarding whether they were fertilized or unfertilized. PINCUS (18) stated that it was extremely difficult to recover unfertilized ova from the uterus and concluded that the eggs were rapidly resorbed or washed out into the vagina. This conclusion was supported by HASHIMOTO (11), who reported that he could not find uterine unfertilized eggs after 152 hours *p. c.* It was, however, demonstrated clearly by ADAMS (1) that a good proportion of unfertilized eggs might persist in the uterus at least up to the 15th day of pseudopregnancy. While the pattern of transport and distribution of rabbit embryos in the oviduct and uterus was studied 15 to 168 hours *p. c.* using HOWE's freezing-clearing

technique by TSUTSUMI and HAFEZ (20). They located unfertilized eggs in the uterus, along with developing blastocysts 168 hours *p. c.*

The present study was conducted to confirm ADAMS' findings and to observe distribution patterns of unfertilized eggs in the uterus of the pseudopregnant rabbits using the freezing-clearing technique.

Materials and Method

Thirty-one Japanese white doe rabbits were mated with vasectomized bucks and injected intravenously with 20 I. U. of HCG to insure ovulation. The does were autopsied 5, 7, 10, 13, 15 and 17 days *p. c.* The wall of the thorax and abdomen was opened and large blood vessels in the thorax were cut immediately after killing the does with an overdose of Nembutal. After removing most of the abdominal intestines, oviducts and uteri were frozen *in situ* quickly, by pouring liquid nitrogen into the abdominal cavity, as described by HOWE (14).

After thawing, the reproductive tracts were excised and each uterine horn, with the adjacent oviduct, was dissected from the fat; this was considered as one unit. The uteri and oviduct were straightened out and pinned through aluminum foil to a supporting board, fixed in AFA, dehydrated in ascending alcohol, bleached by hydrogen peroxide, and cleared in a benzyl-benzoate solution (ORSINI, 17). The number of corpora lutea was counted and the length of oviduct and uterine horn was measured before and after the clearing. A dissecting microscope with transmitted light was used to locate the eggs in the cleared oviduct and uterus.

Results

1. Detectability of eggs

The number of uterine horn in which eggs are located at various stages of pseudopregnancy is as shown in Table 1. Most of uterine horns at 5 and 7 days *p. c.* held eggs, whereas no eggs were located in 27 (68%) out of 40 uterine horns 10 to 17 days *p. c.* Detectability of eggs in each stage is given in Table 2. In general, cleared uterine horns were transparent and clear and showed a yellow appearance. The location of eggs was clear at 5, 7 and 17 days *p. c.* (Figs. 2 and 3). In spite of the same clearing treatment, however, the some uterine horns at 10 days *p. c.* became dark yellow in the mucosa, but were still transparent (Fig. 5). At 13 and 15 days *p. c.* most of uterine horns were dark yellow in the mucosa and contained opaque brown mucus in the uterine lumen. It was noted at times that the brown mucus oozed out into the vagina through the cervix.

TABLE 1. Number of oviducts or uterine horns in which eggs were located at varied days *post coitum*

Days <i>post</i> <i>coitum</i>	Total no. of uterine horns	No. of oviducts or uterine horns		
		Eggs appeared in oviduct	Eggs appeared in uterine horn	No eggs appeared both in oviduct or uterine horn
5	14	2	13 (92.8)*	1 (7.1)*
7	8	0	8 (100.0)	0 (0)
10	10	1	4 (40.0)	6 (60.0)
13	8	1	2 (25.0)	5 (62.5)
15	16	1	4 (25.0)	12 (75.0)
17	6	1	2 (33.3)	4 (66.6)
Total or average	62	6	33 (53.2)	28 (45.1)

* percentage.

TABLE 2. Detectability of eggs located in the uterine horn at various stages of pseudopregnancy

Days <i>post</i> <i>coitum</i>	Total no. of corpora lutea	Total no. of eggs detected	Detectability of eggs (%)
5	93	69	74.2
7	52	41	78.8
10	74	11	12.2
13	56	5	8.9
15	102	7	6.9
17	49	3	6.1
Total or average	426	136	31.9

The uterine mucosa returned to a normal state at 17 days *p. c.*, although the brown mucus still remained partly in the uterine lumen (Figs. 6 and 7). Such changes occurred in uterine horns which disturbed the locating of eggs after 10 days *p. c.*

2. Distribution of eggs in the uterine horn

The position of each egg in the uterine horns at various days after copulation are illustrated by the percentage of distance travelled from the uterotubal junction (Fig. 1). Most of the eggs were retained in proximal third of the uterine horn 5 days *p. c.* However, many eggs (32%) were located around the internal os of the cervix 7 days *p. c.*, while some of

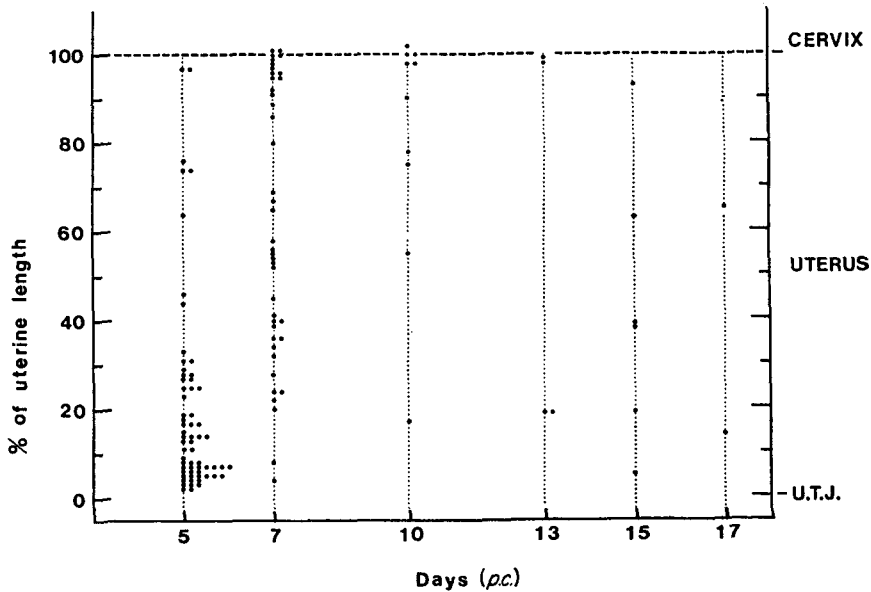


Fig. 1. Position of the rabbit eggs located in the uteri at varied days *post coitum* during pseudopregnancy. The positions of each egg were expressed in percentage of distance travelled in the length of uterine horn.

them were found in the cervix. Although only ten eggs were found in the uterine horns in 3 out of 5 does 10 days *p. c.*, a similar distribution pattern of eggs with that in 7 days *p. c.* is evident. It was impossible to summarize the pattern of distribution of eggs in uterine horns 13, 15 and 17 days *p. c.* because of the limited number of eggs located. It seemed, however, that there was no special tendency in the distribution pattern of eggs within the limit of these days. In Tables 3 and 4, the mean value of uterine length is expressed in percentage of the distance travelled by

TABLE 3. Mean positions of eggs in uterine horns, illustrated in percentage of length of the uterine horn, 5, 7 and 10 days *p. c.*

Days <i>post coitum</i>	Distance travelled by eggs		
	Mean	Standard deviation	Standard error
5	20.3	22.5	2.84
7	61.4	30.5	4.76
10	81.1	26.9	8.48

TABLE 4. Location of eggs closest to uterotubal junction (proximal-most) or nearest to cervix (distal-most) in the uterine horn 5, 7 and 10 days *p.c.* Their positions are presented in percentage of length of uterine horn from proximal to distal end

Days <i>post coitum</i>	Proximal-most Mean (range)	Distal-most Mean (range)	Mean distance between proximal-most and distal- most eggs Mean (range)
5	11(2-64)	39(8-97)	28(1-93)
7	36(4-95)	89(58-101)	52(5-88)
10	50(17-75)	100(98-102)	50(25-81)

eggs and mean locations of the proximal-most and distal-most eggs in percentage of uterine length 5, 7 and 10 days *p.c.* are given. The mean positions of eggs in the uterine horn changed from 20% of 5 days *p.c.* to 81% of 10 days *p.c.*, while the mean position of eggs should approach to the 50% point of length of the uterine horn by spacing of eggs during pregnancy. Also, the proximal-most eggs in the uterine horn should remain near the uterotubal junction throughout the pregnancy, while the proximal-most eggs moved rapidly towards the distal end of the uterine horn. Although these conditions seems to imply rapid transport of eggs in the uterine lumen and expulsion of eggs through the cervical canal; this however is not conclusive because the cleared uterine horns at 13, 15 and 17 days *p.c.* were unsuitable for observation of eggs and some eggs were still located in the uterine horns.

Discussion

Progestational proliferation of the rabbit uterine mucosa during pseudopregnancy is a special phenomenon and reaches its highest level from the fourth to the sixth day after coitus. The maximal branching of the uterine folds occurs at 4 to 9 days *p.c.* The uterine epithelium appears to have been transformed into a syncytium at 8 days *p.c.* and the epithelial cells are sloughed off into the uterine lumen at 9 days *p.c.* The changes in the syncytial epithelium are more advanced and some of them are seen sloughing off at 11 days *p.c.* After 15 days *p.c.* the characteristics of the endometrium in pseudopregnancy are suddenly lost, although there are individual differences. Subepithelial capillary plexus increases remarkably in thickness and the capillaries among uterine glands are stretched lengthwise during pseudopregnancy. Such changes reach a maximum at 6 to 7

days *p. c.* After that the capillaries become gradually short and tortuous (TSUTSUMI and HAFEZ, 19). HAMMOND and MARSHALL (10) noted that the endometrium became congested at the end of pseudopregnancy and blood may have been extravasated in the uterine mucosa. Three stages have been distinguished in cellular contents and state of mucus of the vaginal lumen during pseudopregnancy (TSUTSUMI and HACHINOHE, 21). Stage 3 beginning on the sixteenth day after mating is marked by the reappearance of mucus, either colored or colorless, containing a variety of cell types; leucocytes, erythrocytes, round and columnar epithelial cells, multinucleate giant or syncytial cells, cell masses of ciliated cells and cell debris. These cells may have been derived from the uterine lumen. It is, therefore, evident that the uterine lumen in the pseudopregnant does are filled with various kinds of cells, to such an extent that locating of the unfertilized eggs in the uterine lumen during later half of pseudopregnancy is disturbed and gave rises to a very low detectability of eggs at 10 to 15 days *p. c.* in the present study. Low detectability of eggs at 17 days *p. c.* may suggest that most of unfertilized eggs have already disappeared from the uterine lumen.

However, it is worthy of note that 2 unfertilized eggs were located in both uterine horns of a doe at 17 days *p. c.* in the present observations, although ADAMS (1) recovered 6 unfertilized eggs (recovery rate 20.7%) by flushing physiological saline solution through the uterine horns of 3 pseudopregnant does at 15 days *p. c.* In spite of higher rates of detectability of eggs at 5 and 7 days *p. c.* in the present study as compared with recovery rates of eggs by ADAMS (1), the present rates of detectability of eggs (Table 1) are very low compared with that of pregnant does (almost 100% by TSUTSUMI and HAFEZ, 20). Thus a possibility exists that some of the unfertilized eggs may escape from the uterine lumen to the vaginal lumen during the early days of pseudopregnancy. Hence, very low percentages of detection of eggs are seen at 10, 13 and 15 days *p. c.*, compared with the recovery percentages by ADAMS (1). This may be caused by uterine mucosal changes during pseudopregnancy from which difficulty to locate eggs arises and also by a loss of eggs from the uterine lumen.

Distribution and spacing of rabbit blastocysts in the uterus have been reported by BÖVING (2, 3, 4, 5, 6, 7 and 8) and TSUTSUMI and HAFEZ (20). It was noted that in three uterine horns of two does out of six, seven blastocysts appeared closely adjacent to the internal os of the cervix at 78 hours *p. c.*, although most blastocysts were distributed in the proximal half of the uterine horn (TSUTSUMI and HAFEZ, 20). A similar

tendency seems to exist in the pseudopregnant does at 5 days *p. c.*, also. Spacing of blastocysts, however, begins 114 hours *p. c.*, the blastocysts in the uterus at 5 days *p. c.* are in the process of even spacing. The pattern of distribution of eggs in pseudopregnant rabbit uterine horns at 5 days *p. c.* bears a striking resemblance to that of pregnant does at 108 hours *p. c.* in a previous study (TSUTSUMI and HAFEZ, 20). FUCHS and BELING (8) noted that plasma progesterone started to rise on day 4 in the pregnant rabbits and was significantly higher than in pseudopregnant does on day 5. They suggested that the maternal ovary recognized the presence of blastocysts prior to implantation in the rabbit. The present results seem to support their suggestion. Even spacing of the blastocysts along the uterine horns was accomplished around 6-7 days *p. c.* While about 32% of unfertilized eggs in pseudopregnant does at 7 days *p. c.* were aggregated in segment 10 of the uterine horn, which divided into ten equal segments starting from the uterotubal junction. It seems that even spacing of eggs in the uterine horn can not be achieved in pseudopregnant does.

Summary

Unfertilized eggs were located in the uterine horn during pseudopregnancy in rabbits using the freezing-clearing technique by ORSINI (17) and HOWE (14). Very low detectability of eggs in the uterine horns was accomplished during 10 to 15 days *post coitum* because of difficulty to locate eggs originated by specialized uterine mucosal changes occurring in the pseudopregnant doe. It was, however, noted that a few unfertilized eggs still remained in the uterus at 17 days *post coitum*. A possibility was suggested that the lower detectability of eggs at 5 and 7 days *post coitum* compared with that of pregnant does may be caused by the escape of eggs from the uterine lumen to the vaginal lumen during the early days of pseudopregnancy. Even spacing of eggs in the uterine horn was not attained in pseudopregnant does.

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PLATE 1**EXPLANATION OF FIGURES**

- 2 Eggs located in the proximal part of uterine horn at 5 days *post coitum*. Arrows show eggs. $\times 10.3$.
- 3 An egg (arrow) located in the cervix at 7 days *post coitum*. $\times 13.8$.
- 4 Two eggs (arrows) located around the internal os of the cervix at 10 days *post coitum*. $\times 9.8$.
- 5 Middle part of uterine horn at 10 days *post coitum*. Uterine mucosa has changed dark. $\times 14.3$.
- 6 An egg (arrow) located in the uterus at 17 days *post coitum*. $\times 8.6$.
- 7 The same egg as in Figure 6. $\times 33$.

