Oestrous behaviour, follicular growth and ovulation during pregnancy in the hare (*Lepus europaeus*)

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Summary. Sexual chase was observed throughout pregnancy in hares permanently housed with a male. The incidence of oestrus followed by mating increased rapidly from Day 34 to day 38. Follicular growth was not interrupted; the mean number of large follicles increased at the end of pregnancy. Ovulation could be induced by hCG at any stage of pregnancy, but only on the last days by mating.

Introduction

Oestrous behaviour and mating during pregnancy are uncommon in rodents, although they have been reported to occur in mice (Crew & Mirskaia, 1930), hamsters (Krehbiel, 1952) and rabbits (Hammond & Marshall, 1952). However, in the pregnant rabbit mating does not induce ovulation as it would in the non-pregnant animal (Adams, 1968). In the hare, mating commonly occurring just before term induces ovulation and leads to superfetation (Hediger, 1948; Martinet, Llegouis & Moret, 1970).

The present investigation was undertaken to study oestrous behaviour in relation to follicular growth and incidence of ovulation in the pregnant hare and to improve our knowledge of the mechanisms leading to superfetation.

Materials and Methods

Animals. Two groups of hares were used. Group 1 animals were born in captivity. When mature, they were housed in pairs (one male and one female) in large 2 m² pens in a room with a regulated light-dark cycle of 16 h light (05:00–21:00h)/24 h. Temperature throughout the year ranged from 12 to 25°C. Dry rabbit pellets, hay and water were given *ad libitum*. Group 2 hares, killed in the fields near Cambridge in February 1975, were used only for the follicular growth study.

Oestrous behaviour. Oestrous behaviour was recorded during 290 pregnancies in 58 females. Sexual chase is very rough and may last up to 24 h; mating, usually taking place during the night, was not often observed. When tufts of hair were found in the cage in the morning, it was assumed that sexual chase had occurred during the night. The tufts were removed as soon as noted. If sexual chase and/or mating were seen during the day, they were also recorded.

Follicular growth. Females at different stages of pregnancy were killed and both ovaries removed; the ovaries were fixed in Bouin's fluid, serially cut into 10 µm sections and stained with haematoxylin and eosin. Every fifth section was scanned, and the largest cross-sections of all follicles greater than 500 µm in diameter were measured with an ocular micrometer.

Two sets of ovaries were available: (1) those from 27 females kept in captivity (Group 1) and

(2) those from 39 females killed in the wild (Group 2). The stage of pregnancy was determined by the date of mating (Group 1: day of mating = Day 0 of pregnancy) or fetal weight (Group 2).

Induction of ovulation. Twelve females at different stages of pregnancy were given an intravenous injection of 100 i.u. hCG in 1 ml saline (9 g NaCl/l). Laparotomies were performed 3 days later under pentobarbitone sodium anaesthesia and both ovaries were examined for the presence of ovulations. Laparotomies were also performed on 9 females 3 days after mating.

Results

Oestrous behaviour

As shown in Text-fig. 1, sexual chase followed by mating was observed from Days 2 to 33; there was no day-to-day frequency variation. From Day 34, oestrous behaviour increased dramatically with a peak on Days 37 and 38, i.e. 3–4 days before parturition.

Text-fig. 1. The frequency of sexual chase and mating recorded during 290 pregnancies in 58 female hares in captivity.

Follicular growth

The follicles, divided into 3 classes according to size, were distinguished as normal or atretic.

Normal follicles. In the 500–999 μm class there was no variation throughout pregnancy. The mean number per female ranged from 30 to 45 in Group 1 and from 55 to 65 in Group 2. The numbers of follicles in the size ranges of 1000–1499 μm in females of Group 1, and of ≥ 1500 μm in females of Groups 1 and 2 increased as pregnancy progressed. The differences were significant only for the largest follicles ($P < 0.01$ in Group 1, $P < 0.05$ in Group 2) (Table 1).

When ovaries with or without corpora lutea from the same females were compared, the number of normal follicles 1000–1499 and ≥ 1500 μm diameter was always greater in ovaries without corpora lutea. The differences were significant for the Group 1 ovaries ($P < 0.05$) (Table 2).

Table 1. Size distribution of normal follicles/female during pregnancy in captive (Group 1) and wild (Group 2) hares

Females		Day of pregnancy	Fetal weight (g)	Follicle size (μm)		
Group	No.			500–900	1000–1499	≥ 1500
1	6	14–18	0.1–1.6	36 $\pm$ 10	4.0 $\pm$ 1.0	0.8 $\pm$ 0.4
	5	20–25	7–20	29 $\pm$ 4	4.8 $\pm$ 1.7	1.8 $\pm$ 0.3
	10	28–35	22–65	31 $\pm$ 6	6.7 $\pm$ 1.7	2.5 $\pm$ 0.4
	6	37–40	≥ 80	46 $\pm$ 5	7.8 $\pm$ 1.2	2.7 $\pm$ 0.2
2	15	—	0.1–2.9	64 $\pm$ 2	3.5 $\pm$ 0.2	2.1 $\pm$ 0.1
	8	—	7–18	66 $\pm$ 3	3.4 $\pm$ 0.5	1.9 $\pm$ 0.2
	10	—	20–52	64 $\pm$ 4	2.9 $\pm$ 0.2	2.7 $\pm$ 0.2
	6	—	≥ 80	55 $\pm$ 3	2.9 $\pm$ 0.5	3.0 $\pm$ 0.2

Values are mean $\pm$ s.e.m.**Table 2.** Size distribution of normal follicles/ovary of hares during pregnancy according to the presence or absence of corpora lutea (CL) in the ovary

Females		Day of pregnancy	Fetal weight (g)	Follicle size			
Group	No.			1000–1499 μm		$\geq 1500 \mu\text{m}$	
				CL	No CL	CL	No CL
1	8	14–25	0.1–20	1.0 $\pm$ 0.3	3.1 $\pm$ 1.1	0.7 $\pm$ 0.4	1.5 $\pm$ 0.5
	9	28–40	22–120	2.5 $\pm$ 0.4	4.4 $\pm$ 0.5	0.6 $\pm$ 0.4	2.1 $\pm$ 0.6
2	18	—	0.1–18	1.1 $\pm$ 0.4	2.6 $\pm$ 0.5	0.7 $\pm$ 0.3	1.3 $\pm$ 0.3
	8	—	20–52	1.2 $\pm$ 0.3	1.6 $\pm$ 0.5	1.3 $\pm$ 0.1	1.6 $\pm$ 0.4
	5	—	≥ 80	1.4 $\pm$ 0.2	1.6 $\pm$ 0.4	1.4 $\pm$ 0.2	1.8 $\pm$ 0.3

Values are mean $\pm$ s.e.m.

Atretic follicles. Two types of atresia were encountered, either (i) a loosening and sloughing of the granulosa cells lining the antrum with the appearance of pyknosis or (ii) a pseudo-luteinization with decreased tinctorial affinity. The first type of atresia was seen mainly in the large follicles (1000–1499 and $\geq 1500 \mu\text{m}$) and the second one in the 500–900 μm diameter follicles. There was no clear variation in the incidence of atresia between follicle classes, pregnancy stages or the 2 groups of females (Table 3).

Table 3. The percentages of atretic follicles in the total number of follicles in each size class in pregnant hares

Females		Day of pregnancy	Fetal weight (g)	Follicle size (μm)		
Group	No.			500–999	1000–1499	≥ 1500
1	6	14–18	0.1–1.6	32	20	58
	5	20–25	7–20	25	22	36
	10	28–35	22–65	31	23	32
	6	37–40	≥ 80	26	13	29
2	15	—	0.1–2.9	35	10	53
	8	—	7–18	26	4	21
	10	—	20–52	27	15	31
	6	—	≥ 80	30	11	20

Induction of ovulation

The injection of hCG during pregnancy always induced ovulation; mating also induced ovulation but only on the last days before parturition (Table 4). Ovulation induced by hCG was followed by abortion 2 or 3 days later if the hCG was given between Days 14 and 28. After Day 32, pregnancy was not interrupted (Table 4).

Table 4. Induction of ovulation during pregnancy in hares

Ovulation induced by	Day of pregnancy	No. of ovulations	Day of abortion	Day of parturition
hCG	14	2	?	
	15	3	18–19	
	17	3	19	
	25	2,1,1	28,28,27	
	28	1,2	30,31	
	33	1,1		40,39
	36	3		39
	37	2		40
Mating	14	0,0		
	23	0		
	26	0		
	35	3,2		38,41
	36	3,1,4		41,40,40

Discussion

Spontaneous superfetation, defined as “ovulation, fertilization and implantation of a second set of eggs during pregnancy” (Rollhauser, 1949), is very rare. Superfetation produced by fertile matings 1–5 days before parturition occurs in wild (Flux, 1967), as well as in captive (Hediger, 1948) hares; 60% of captive female hares mate and conceive at the end of pregnancy (Martinet *et al.*, 1970) and for this sexual receptivity, follicular growth and ovulation must all be possible. The present results show that all 3 of these conditions do occur in the hare; they do not seem to exist concomitantly in other species, except in the hystricomorph rodent, the casiragua (Weir, 1974).

Sexual receptivity throughout pregnancy with increasing duration or intensity on the last days has been reported for the rabbit (Beyer & Rivaud, 1969). In casiragua, 50% of females experience oestrus 24–48 h before parturition (Weir, 1974), a proportion nearly as high as that in the hare.

Follicular growth is not interrupted during pregnancy in the rabbit (Hammond & Marshall, 1925; Hill & Parkes, 1932), the hamster or the mouse (Greenwald, 1964; Greenwald & Choudary, 1969). The Graafian follicles are present at any stage of pregnancy in the hare, and their number increases as pregnancy progresses, as in the rabbit (Adams, 1968). The small increase reported in the wild females may be due to the lack of precision in determining the stage of pregnancy by fetal weight. The fact that the number of large follicles is always higher in ovaries without corpora lutea is related to the tendency for the ova to be shed by alternate ovaries (Flux, 1967). The differences in follicle number between captive and wild hares may be attributed to several causes. In captive females housed under standard long daylengths (16L:8D) all year round, there is no seasonal variation in the breeding pattern (Martinet, 1976), but the wild hares were killed in February when the daylength is increasing. All the captives hares were multiparous, while nothing was known about pregnancies, if any, in the wild hares.

In all the species cited above, as in the hare, ovulation can be induced by exogenous gonadotrophins during pregnancy. In the hare, ovulation can also be induced by mating just before term when plasma progesterone levels are usually decreasing (Caillol & Martinet, 1976; Stavy, Terkel & Kohen, 1978), although they are sometimes still high (M. Caillol, unpublished data). The fertile prepartum matings in the casiragua (Weir, 1974), occur when progesterone values in the peripheral blood are still high (R. B. Heap, personal communication). Information on hypothalamic sensitivity to the steroidal feedback in the hare is required to extend understanding of the mechanisms leading to superfetation.

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