EFFECTS OF CONCURRENT PREGNANCY AND LACTATION IN RABBIT DOES ON THE GROWTH OF FOLLICLES IN DAUGHTERS' OVARIES

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ABSTRACT : The aim of this experiment was to study the effects of concurrent pregnancy and lactation in the mother on follicular development in daughter rabbits. Young females from mothers lactating (group PL) or not (group P) during gestation were sacrificed on days 29 ($n_{PL} = n_P = 5$), 49 ($n_{PL} = n_P = 5$) or 70 ($n_{PL} = 4$, $n_P = 5$) and one ovary was taken from each. Follicles were divided into size bands according to their diameter. The P and PL group counts in each band were similar at 29 and 49 days, but at 70 days the number of larger growing follicles (diameter > 241 μm) was lower in the PL than in the P does (83 vs. 155; $P<0.05$). A mathematical model for follicular development was fitted to the size-class frequencies for animals in both groups and showed that the duration of the first growth phase was longer in the PL than in the P group (26.6 days vs. 14.9 days; $P<0.001$). These results suggest that concurrent lactation during pregnancy in primiparous does significantly affects early follicular development and delays the appearance of larger ovarian follicles in the daughters.

RESUME : Effets de la simultanéité de la gestation et de la lactation chez la mère sur la croissance folliculaire chez les jeunes lapines
L'objectif de cette expérience est d'étudier les effets de la simultanéité de la gestation et de la lactation chez la mère sur la croissance folliculaire chez les jeunes lapines. Des jeunes lapines provenant de mères allaitantes (groupe PL) ou non (groupe P) pendant la gestation ont été sacrifiées à 29 ($n_{PL} = n_P = 5$), 49 ($n_{PL} = n_P = 5$) ou 70 jours d'âge ($n_{PL} = 4$, $n_P = 5$) afin de prélever un des deux ovaires. Les follicules ont été répartis en classes de taille en fonction de leur diamètre. Le nombre de follicules dans chaque classe était similaire dans les deux groupes P et PL à 29 et 49 jours d'âge, mais à 70 jours le nombre de follicules de grande taille (diamètre > 241 μm) était plus faible dans le groupe PL que dans le groupe P (83 vs. 155; $P<0,05$). Un modèle mathématique décrivant la croissance folliculaire a été ajusté aux données de taille des follicules obtenues à chaque stade d'abattage et montre que la durée de la première phase de croissance est plus longue chez les femelles du groupe PL que chez celles du groupe P (26,6 jours vs. 14,9 jours; $P<0,001$). Ces résultats suggèrent que la simultanéité de la gestation et de la lactation chez la lapine primipare affecte le développement folliculaire précoce et l'apparition des follicules pré-ovulatoires chez les jeunes lapines.
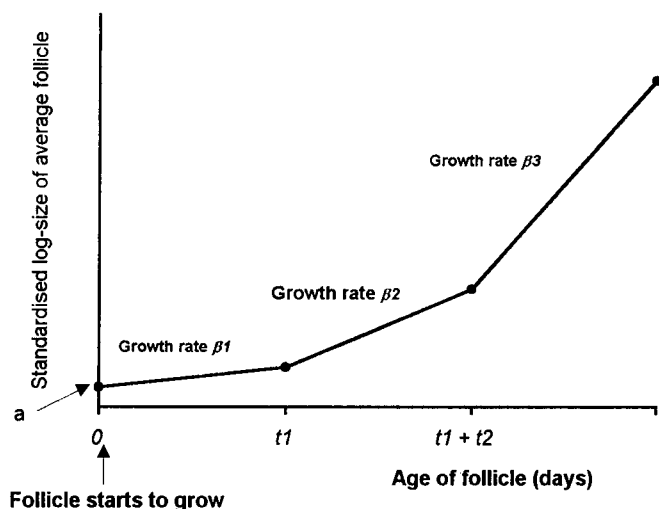
INTRODUCTION

Female rabbits may be fertilised shortly after parturition, and be simultaneously pregnant and lactating. However, the voluntary feed intake of primiparous does is insufficient to supply all the nutritional requirements for maternal tissue growth, foetal development and milk production (MAERTENS and DE GROOTE, 1988; FORTUN-LAMOTHE and LEBAS, 1996). As a consequence, concurrent pregnancy and lactation induces competition between the mammary glands and the pregnant uterus for the supply of nutrients. Therefore, when the mother is simultaneously pregnant and lactating, foetuses are exposed to undernutrition. This situation is detrimental to foetal growth which is reduced in lactating does (-19.6% on day 28 of pregnancy ; FORTUN et al., 1993) and leads to rabbits which are lighter at birth (-2.1% ; FORTUN-LAMOTHE et al., 1999).
In rabbits, the pool of germinal cells available during reproductive life is established during foetal life

(maximum 26-28 days post coïtum ; CHRÉTIEN, 1966) and until 10 days after birth (PETERS et al., 1965; MAULÉON and MARIANA, 1977). In this species, by 30 days after birth all follicles are in the same stage of development and the initiation of growth can be seen in the proliferation of follicular cells and the enlargement of the oocyte (MARIANA and DE POL, 1986). There is a continuing recruitment of growing follicles, and many cycles of follicle growth are progressively superposed. In view of this, the quantitative study of follicular development calls for an attempt to model follicular growth. Two approaches to modelling may be distinguished: i) compartmentalisation of the growth process with estimation of the time spent in each growth compartment (FADDY et al., 1976), and, alternatively, ii) attempting to describe the growth process for individual independent follicles with due regard to the qualitative development of follicles over time. In this second approach, it must be noted that each follicle is of necessity observed only once. However, under suitable assumptions the distributions of follicle numbers and sizes observed in different animals at varying ages yields useful quantitative information about the growth of ovarian follicles. The form of model and analysis used here enables such inferences to be made,

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Growth rate $\beta3$

Growth rate $\beta2$

Growth rate $\beta1$

Standardised log-size of average follicle

a

0

t1

t1 + t2

Follicle starts to grow

Age of follicle (days)

Figure 1 : Schematic description of three-phase follicle growth model.

coherently over animals aged from 29 to 70 days and consistently with an extensive body of numerical data. READ *et al.* (1981) have applied the second approach to rats, but not in the setting of a comparative trial.

It has been shown that size and/or number of growing follicles were reduced if the females are subjected to food restriction just before or after puberty (PRINTZ and GREENWALD, 1970; LINTERN-MOORE *et al.*, 1981; HULOT *et al.*, 1982). Additionally, BORWICK *et al.* (1997) have shown in the ewe, that undernutrition of the mother during pregnancy retards ovarian development in foetus. However, the effects of undernutrition during early life on follicular development in the rabbit are little known. Therefore, the aim of this experiment was to study the effects of concurrent pregnancy and lactation in rabbit does on the growth of follicles between 30 and 70 days after birth in young females. A quantitative three-phase model for follicular growth in the form of approach ii) above was developed and successfully fitted to the data.

MATERIALS AND METHODS

1. Animals

Fourteen 24-week-old female rabbits (from the INRA crossbred line A1067) were assigned to one of the two groups for study. In the PL group, females were mated within 24h after parturition. They were allowed to suckle their litter, and so in this group experimental pregnancy was concurrent with lactation: the daughters from these pregnancies concurrent with lactation constituted the PL group. To establish the control (P)

group, females, matched pairwise with the PL females, were not remated after parturition and their daughters (from a pregnancy not concurrent with lactation) served as controls. In both groups weaning occurred on day 28 of lactation. All animals (mothers and young) had free access to water and to a commercial diet (17.5% of crude protein, 2330 kcal digestible energy per kilogram of feed), and were subject to a controlled light/dark diurnal cycle (16h/8h). The does were caged individually, whereas the growing rabbits were reared collectively after weaning.

The young females (14 and 15 in the PL and P groups respectively) were weighed individually each week from birth (day 0) to sacrifice on days 29 (5 PL and 5 P), 49 (5 PL and 5 P) or 70 (4 PL and 5P). Ovaries were removed after sacrifice and stored in Bouin Hollande solution pending histological study.

2. Histology

The ovaries were placed in paraffin and then serially sectioned in 5μm slices. All the sections were mounted and coloured with Hemalun which permitted normal and atretic follicles to be distinguished. Only one of the two ovaries was used to count follicles as it is known that the numbers of follicles in corresponding stages of growth in the two ovaries are highly correlated (MARIANA and DERVIN, 1992). The average diameter of each follicle was measured from the section including the oocyte nucleus.

Only follicles larger than 108μm were counted, since we had previously shown that smaller follicles did not initiate growth by simultaneous enlargement of the oocyte and proliferation of follicular cells (MARIANA *et al.*, 1989). Follicles were assigned to four broad qualitative size bands. In the first of these, follicles having diameters between 109 and 241μm and typically 2, 3 or 4 layers of cells around the oocyte. The second band contains follicles with diameters between 242 and 472μm and 4 or 5 layers of cells. The third band includes 'large' follicles with a unified antrum and diameters in the range 473-800μm, and the fourth band consists of 'very large' follicles greater than 800μm in diameter which can ovulate if stimulated by LH (HULOT and MARIANA, 1985). These four broad bands were subdivided into a much finer classification for the purpose of quantitative analysis and model fitting with boundaries given by: 108.5μm, 141.5μm, 174.5 μm, and so on at intervals of 33μm up to 966.5μm; 1033.5μm, 1150μm, 1250μm, and so on at intervals of 100μm up to 2050μm; and > 2050μm.

3. Construction of the model

A quantitative model was developed to describe the growth of follicles on the basis of measured follicular diameters in the ovaries of rabbits sacrificed at 29, 49 and 70 days. Previous results have suggested that follicle growth can be represented in terms of three main phases designated 'slow', 'intermediate' and 'fast' respectively (PEDERSEN, 1969; HAGE et al., 1978; MARIANA et al., 1989). At least in the present work, it appears that growth in each phase can be assumed to be exponential in actual size (diameter), or, equivalently, linear with time in the logarithm of size. The resulting piecewise linear form of growth on a logarithmic scale is shown in Fig. 1. In modelling growth, it is therefore convenient to work with the (standardised) logarithm of (actual size / 108.5) which thus increases piecewise linearly with time from a minimum possible value of zero: we call this quantity 'standardised log-size'. On this basis we arrive at a model in terms of 8 parameters, each of which has a clear physiological interpretation: β_1, β_2 and β_3, representing the growth rates in each of the three main phases; t_0, the time after birth at which the first follicles start to grow; t_1 and t_2, the respective duration of the first two phases of growth; c, the constant rate of recruitment to the growing population of follicles; and a, the average initial standardised log-size of a follicle, it being assumed that the initial standardised log-sizes are exponentially distributed. It might be argued that a quadratic or other smooth curve effectively equivalent to the piecewise linear plot could be defined using fewer parameters. However, such a representation would not correspond to the three phase model suggested by times of qualitative changes in hormonal activity (MARIANA et al., 1989; HULOT et al., 1990). In particular, the duration and growth rates in the first two phases would not be clearly identified. Due to lack of data and the nature of the experimental design, it was not found possible to estimate c and t_0, even globally, using the above model. Instead, these parameters are estimated by suitably weighted regressions of the total observed frequencies of growing follicles on age of animal within the P and PL groups. The general model defined in the Mathematical Appendix provides for the loss of follicles due to atresia. However, as no atresia was observed in this study up to 70 days it was not possible to identify any rate of loss to this cause and so the probability of surviving atresia was set equal to 1.

4. Statistical analyses

All data were first subjected to analysis of variance (GLM) using the SAS (SAS, 1990) package. For weight at the time of sacrifice, the treatment groups P and PL comprised the main effects of the model. The numbers of follicles in each of the main size bands at each age of sacrifice were analysed similarly adding to the previous model the live weight of rabbits at birth as a covariate. Finally, the mathematical model described above was fitted to the control and treatment groups of animals, both separately and collectively, with the aims of estimating 'global' or population average parameter values to characterise the two groups and testing for between-group differences. Parameter estimation was carried out using the method of Maximum Likelihood and employing the simplex method of function minimisation developed by NELDER and MEAD (1965). A formal comparison of the control and treated groups was made using the asymptotic generalised likelihood-ratio test. A rough test of the equality of the values of each model parameter in the two groups was made by comparing the difference between these values to an asymptotic estimate of its standard error in each case.

RESULTS

1. Post-natal growth of the young

The birthweights of daughters were on average about 9% lower in the PL group (49.9 ± 2.9 g compared to 54.7 ± 3.5 g in the P group) but this difference is not significant. From birth to 70 days (2132g ± 102 g and 2171g ± 147 g in the PL and P groups respectively) the weights in the two groups are not significantly different, but at every time the mean in the PL group is less. To identify the suggested small reduction in weight in the PL group in the early stages of life with reasonable certainty would require larger numbers of animals than were used in this study.

2. Number of follicles in each class

The numbers of follicles in bands 1, 2, 3 and 4 at each sacrificial stage in the PL and P groups are summarised in Table 1. In both groups the numbers of small follicles (band 1) increase with age ($P < 0.01$); at 29 days only small follicles are seen, and large follicles (bands 3 and 4) appear only at 70 days. The groups do not differ significantly in their average numbers of band 1 and band 2 follicles at 29 days and 49 days. At 70 days, the numbers of small follicles were 41% higher in females of the PL group than in the P group ($P = 0.066$) and the numbers of follicles larger than 241 μm (bands 2, 3 and 4) were 46% lower in PL than in P females ($P = 0.039$).

3. Modelling follicular growth: comparisons between treatment groups

In order to be useful, a mathematical model must provide a satisfactory fit to the data. In addition, the parameters of the model should be in few number, should be reliably estimated (i.e. with relatively small standard errors) and should be easily interpreted in

Table 1 : Numbers of ovarian follicles in each size band at 29, 49 and 70 days in females born to does which were lactating (PL group) or not lactating (P group) during pregnancy

| Size Band		1	2	3	4
Diameter		109-241μm	242-472μm	473-800μm	> 800μm
	No. of females	Mean (S.D.)	Mean (S.D.)	Mean (S.D.)	Mean (S.D.)
Age at slaughter					
29 days PL group	5	50 (41)	-	-	-
P group	5	62 (70)	-	-	-
P-value		0.70	-	-	-
49 days PL group	5	464 (289)	4 (7)	-	-
P group	5	550 (165)	7 (11)	-	-
P-value		0.64	0.68	-	-
70 days PL group	4	1250 (687)	70 (29)	8 (6)	5 (4)
P group	5	889 (191)	127 (57)	19 (16)	9 (6)
P-value		0.066	0.039	0.10	0.058

P-values are for analyses of covariance using weight at birth as a covariate.

physical or biological terms. With the introduction of some individual values specific to each animal, the 8-parameter mathematical model defined above successfully fitted the data (P-value for χ^2 goodness-of-fit test > 0.05) in almost all cases. Moreover, all 8 model parameters have a clear meaning. For brevity and simplicity, only the global (estimated population average) values are given here, the details of the animal-specific values being omitted. The success of the 8-parameter model in fitting the data justifies its use as a basis for further tests of treatment group differences.

The test used for differences between the P and PL groups is the asymptotic χ^2 generalised likelihood-ratio test, which is based on maximum likelihood analyses of the data conducted i) separately for each group and ii) for the two groups combined. This overall test finds decisively in favour of a significant difference between the two groups (*P*<0.001), and so further discussion is based on the parameter estimates obtained by fitting the follicular growth model to the P and PL data separately. It must be noted that this highly significant between-groups difference is the result of a far more powerful and sensitive analysis than the crude comparisons of the proportions of follicles in the four broad size bands (each spanning many days of follicular growth) described in section 3.2. In order to fit the mathematical model, follicles were classified into no fewer than 38 size groups (rather than just four), thereby using far more of the information contained in the original data. Estimates of the global model parameters, as fitted to

the full set of 15 control (P) and to the 14 experimental (PL) animals, are shown in Table II. Estimates of the time of initiation of growth (t_{0P} = 27.45, t_{0PL} = 28.02 days), recruitment rate (c_P = 24.58, c_P = 25.11 follicles per day), the average initial log-size (a_P = 0.158, a_{PL} = 0.149) and the initial growth rate (β_{1P} = 0.00751, β_{1PL} = 0.00831) are similar in the two groups. The effective equality of these four 'early development' parameters is entirely consistent with the similarity of the PL and P size band numbers at 29 and 49 days as noted above. These results also suggest that at 29 days and 49 days, follicle growth in both groups of animals might be adequately described by a single growth rate model, whilst at 70 days the full three stage model described here is required to fit the data in both groups. Consequently, significant differences between the groups are found in regard to the parameters β_2, β_3, t_1 and t_2. Thus, the duration of the slower initial growth phase (t_1) is much longer (26.62 ± 1.20 vs. 14.88 ± 2.41), and that of the second stage (t_2) is much shorter (13.61 ± 1.36 vs. 25.65 ± 2.75) in the PL than in the P group (*P* < 0.001). On the other hand, the second and third growth rates (β_2 and β_3) are larger in the PL than in the P group (*P* = 0.016 for both). Since in the PL group the follicles spend 12 days longer in the slow first growth stage than do follicles in the P group, it would be expected that the median follicle size for an animal in the experimental (PL) group would be less than that for a control (P) animal of the same age, and similarly for other quantiles, throughout the age-range of observation (29 to 70 days). Clear confirmation of this statement is provided by the time-plots of corresponding quantiles (10%, 25% (lower quartile), 50% (median), 75% (upper quartile) and 90% points) of the distribution of follicle size for animals in the two groups which are shown in Fig. 2. and by the fact there are more follicles in band 1 in the PL than in the P group (Table I). The compensating higher later growth rates in PL seem to reduce this gap for larger follicles (upper quartile and 90% points) in 70-day animals, but this feature should be viewed with caution as β_3 in PL group is estimated with a very high standard error.
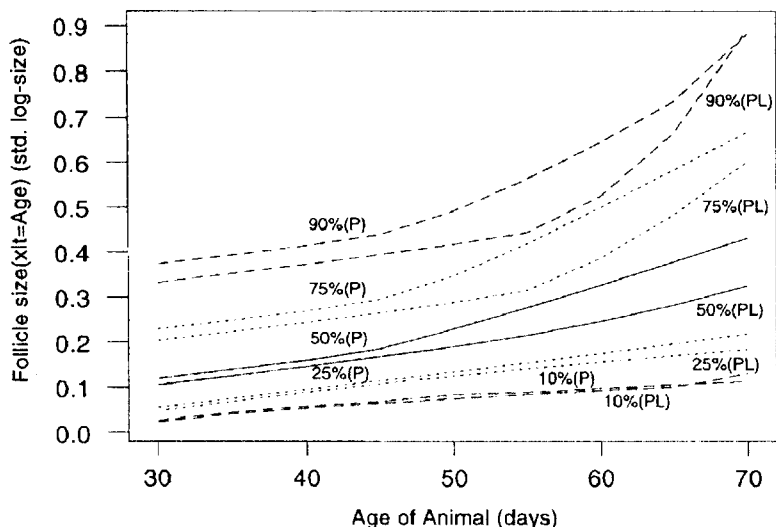
Follicular diameters (μm) corresponding to standardised log-sizes 0.1, 0.2,..., 0.9 are as follows:

Std. Log-size	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
Diameter (μm)	120	133	146	162	179	198	218	241	267

Fig. 2: Increase of quantiles of follicle size with time in females born to mothers which were lactating (PL group) or not lactating (P group) during pregnancy.

The 10%, 25%, 50% , 75% and 90% quantile points of the distribution of standardised log-size are obtained by numerical inversion of the cumulative distribution function computed for animals from 29 to 70 days of age at 5-day intervals.

DISCUSSION

FORTUN et al. (1993) have previously shown that foetal growth was reduced in lactating compared to non-lactating does, due to competition between the mammary glands and the pregnant uterus for available nutrients. In the present study, the differences in the birth weights of rabbits in the two groups were not significant, possibly due to the small numbers of animals used. In the same way, after birth the growth of the young was not significantly affected by whether or not their mothers were lactating during pregnancy. Nevertheless, the body composition of new-born rabbits could have been affected by concurrent lactation during foetal life as shown by FORTUN-LAMOTHE et al. (1999).

In agreement with previous results (MARIANA and DE POL, 1986), at 29 days after birth it was found that most ovarian follicles were primordial, with only a few larger than 100μm. Further deductions can be made in the light of the findings available from a new mathematical model for follicular development in terms of three main phases of growth. The results of this work showed that the rate and time of growth initiation (c and t_0) and the first phase growth rate (β_1) are

not affected by the experimental treatment. These results suggest that the initiation and first phase of follicular growth in the daughters are little changed by concurrent lactation during pregnancy. On the other hand, the markedly longer duration of the first phase (t_1) and the higher second phase growth rate (β_2) in the PL group suggest that the rise of gonadotrophins occurs later in daughters whose mothers were lactating during pregnancy. Indeed, in rabbit species, the rise of follicle stimulating hormone associated with the formation of antral follicles occurs 6-7 weeks after birth (HULOT et al., 1990). The finding of a higher third growth rate parameter (β_3) in the experimental group should be viewed with caution, as its significance could merely reflect the very small but highly variable numbers of large follicles (band 4, > 800μm) at 70 days. It is also possible that the imminent, albeit unobserved, atresia of some of the largest follicles could explain such variability, since cell proliferation and growth are reduced with the onset of atresia (MARIANA and DERVIN, 1992). Actually, the atresia appears only at about 11 weeks of age in the rabbit (HULOT et al., 1982), which is why we failed to observe atresia in the ovaries at the 3 stages of slaughter. Moreover, HULOT

Table 2 : Estimates of parameters of follicle growth model for females born to mothers which were lactating (PL group) or not (P group) during pregnancy

Parameter*	P Group (Control) Estimate (standard error)	PL Group (Experimental) Estimate (standard error)	Comparison P-value
a^1 (μm)	0.158 (0.007)	0.149 (0.007)	0.347
β_1	0.00751 (0.00089)	0.00813 (0.00054)	0.552
β_2	0.0232 (0.0014)	0.0401 (0.0069)	0.016
β_3	1.06 (0.21)	3.59 (1.03)	0.016
t_0 (days)	27.45 (1.33)	28.02 (0.89)	0.722
t_1 (days)	14.88 (2.41)	26.62 (1.20)	0.00002
t_2 (days)	25.65 (2.75)	13.61 (1.36)	0.00009
c^2 (no. per day)	24.58 (2.35)	25.11 (5.25)	0.939

*See text for full definitions of the model parameters
[1] average starting standardized log-size
The actual average starting size (follicular diameter) is 108.5 e$^{0.158}$ = 127.1μm for the control group and 108.5 e$^{0.149}$ = 125.9μm for the experimental group
[2] number of follicles per day

et al. (1990) found an increase in luteinizing hormone at 70 days, suggesting that the third growth rate may well be relevant to our data although it was not investigated in this study.

GONDRET *et al.* (1997) have previously shown that concurrent pregnancy and lactation delays the myofibrillar maturation rate of the young. The present results indicate that concurrent pregnancy and lactation in the does also affects follicular development in the daughters. The two hypotheses that could explain this result are that concurrent lactation is responsible for modification of the hormonal environment of the pregnant does and/or that undernutrition during early life impairs the follicular development of young females.

BORWICK *et al.* (1997) have shown that undernutrition of the ewe from the time of mating retards development in foetal ovaries in mid-gestation. LINTERN-MOORE *et al.* (1981) have shown that a food restriction (-50%) from day 21 to day 42 of life reduced the number of growing follicles in pre-pubertal rats. In the same way, PRINTZ and GREENWALD (1970) observed a reduction in the numbers of large follicles together with anovulation in adult hamsters submitted to 9 days of starvation. Finally, the frequency of ovulation, size and number of large follicles were lowered in rabbit does under feed-restriction between 11 and 20 weeks of age (-75% of control animals; HULOT *et al.*, 1982). Undernutrition during early life could therefore explain the higher number of primary follicles and the lower number of growing follicles in young females born from simultaneously pregnant and lactating rabbit does.

Lactation modifies the progesterone, oxytocine and prolactin levels of pregnant does (FUCHS *et al.*, 1984; FORTUN *et al.*, 1993). However, the effects of these changes on folliculogenesis remain to be demonstrated.

We believe this to be the first study of follicle growth in which a mathematical model is used to evaluate quantitatively and coherently the effect of a treatment on the growth of follicles. In a quantitative and coherent analysis, we assume a mathematical law for the growth of a follicle, together with a probabilistic mechanism for variation, with which the entire data from one animal or from many are reconciled in the statistical derivation of mutually consistent estimates. A well-defined probability distribution for the sizes of follicles in an animal of given age is a necessary basis for fitting growth models that are clearly consistent with both the average trends and the variability represented in the data. Various purely descriptive methods that have in the past been used to establish growth curves from size-band counts of follicles without such a basis would therefore seem to be less coherent or satisfactory from this point of view. A mathematical model for follicular growth enables us to quantify in terms of its estimated parameters, and test for significance, the

effects of treatments such as hormonal implementation or nutritional modification. When the model parameters have physiological meaning, as they clearly do here, there may be implications (such as the longer duration of the slow growth phase in the experimental group) to which the earlier methods of analysis give no obvious access. The form of model used here is presented as exploratory rather than ideal; however, it provides a successful fit to a complex set of observations and, given further data, would be capable of suitable refinement.

CONCLUSION

Our results suggest that concurrent lactation during pregnancy in primiparous does affects early follicular development and delays appearance of larger ovarian follicles in the daughters. The mechanisms implicated in these effects remain to be elucidated, as do the consequences for subsequent reproductive life. Use of a mathematical model for follicular development is found greatly to improve the precision and power of the findings.

MATHEMATICAL APPENDIX

The purpose of this Appendix is to provide a mathematical definition of the ovarian follicle growth model in terms of the eight parameters c, t_0, a, β_1, t_1, β_2, t_2 and β_3. Let $f(w)$ and $F(w)$ respectively denote the probability density function and cumulative distribution function of the standardised logsize, w say, of a follicle when it starts to grow.

Let $s = G(u)$ be the growth function of a follicle, that is, the increase in standardised logsize of a follicle that has been growing for a time u.

Let $u = G^{-1}(s)$ be the inverse function of G; then u is the time taken by a follicle to grow from a standardised logsize of w to a standardised logsize of $s+w$.

In general, ovarian follicles may be subject to atresia. Let $Q(u)$ be the probability that a given follicle survives a time period of growth of at least u before becoming atretic.

Let $M(x|t)$ denote the cumulative distribution function of the standardised logsize x of a given growing follicle in an animal of age t.

Then

$$M(x\,|\,t) = \frac{1}{c(t-t_0)} \int_0^{\min(t-t_0,\,G^{-1}(x))} cQ(u)F(x-G(u))du,$$

where $c(t-t_0)$ is the total number of follicles recruited (at a constant rate of c per unit time) to the growing population in an animal aged t in which the growth process of ovarian follicles started at age t_0. This formula represents the probability that a randomly chosen growing follicle in an animal of age t has standardised logsize $\leq x$. The numerator is a count of all growing follicles with standardised logsize $\leq x$ that have survived to time t and is divided by $c(t-t_0)$ in the denominator, which is the total number of follicles that have started to grow since time t_0. To construct the numerator, suppose that a typical follicle has been growing for a time u, then the chance that it is still alive (i.e. is not dead or atretic) is $Q(u)$. Also, if its standardised logsize after a time u is at most x, then its starting size must have been at most $x-G(u)$, and the probability of this is $F(x-G(u))$. The number of follicles that started to grow during the small time interval $(t-u, t-u+du)$ is cdu, and so $Q(u)F(x-G(u))cdu$ is the probability that follicles that started to grow during the small time interval $(t-u, t-u+du)$ are observed still growing at time t with standardised logsize $\leq x$. Finally, we sum (integrate) this probability over all possible values of u, that is, from 0 to the minimum of $(t-t_0)$ and $G^{-1}(x)$, noting that a follicle that has been growing for longer than $G^{-1}(x)$ will necessarily have standardised logsize $> x$, i.e. will be too large to be counted. If it is assumed or known experimentally that ovarian follicles are not subject to atresia in the animals under study, then the function $Q(u)$ is set equal to 1 and the formula for $M(x|t)$ simplifies to

$$M(x \mid t) = \frac{1}{(t-t_0)} \int_0^{\min\left(t-t_0, G^{-1}(x)\right)} F(x - G(u)) du$$

For convenient choices of the functions F, G and if necessary Q, $M(x|t)$, and hence the probability that a given growing follicle occupies any specified size band, can be expressed in simple closed form. In this paper, the probability density function of the standardised logsize, w say, of a follicle when it starts to grow is assumed to be exponentially distributed, so that

$$f(w) = \frac{1}{a} \exp\left(-\frac{w}{a}\right), \quad w > 0,$$

where a, the mean of this distribution, therefore denotes the population average value of the standardised logsize of follicles when they start to grow.

The follicular growth function $s = G(u)$ is assumed to have the 3-phase piecewise linear form

$$G(u) = \beta_1 u, \quad 0 < u < t_1;$$
$$= \beta_1 t_1 + \beta_2(u - t_1), \quad t_1 \leq u < t_1 + t_2;$$
$$= \beta_1 t_1 + \beta_2 t_2 + \beta_3(u - t_1 - t_2), \quad u \geq t_1 + t_2,$$

where s denotes the increase in standardised logsize of the follicle if it has been growing for a time u. Thus the first phase of growth is at rate β_1 for time t_1, the second phase is at rate β_2 for a further time t_2 and the third phase is at rate β_3 which applies for follicles observed in animals aged at least up to 70 days that have been growing for longer than $t_1 + t_2$. The inverse function for this choice of G is given by

$$G^{-1}(s) = \frac{s}{\beta_1}, \quad 0 < s \leq \beta_1 t_1;$$
$$= t_1 + \frac{s - \beta_1 t_1}{\beta_2}, \quad \beta_1 t_1 < s \leq \beta_1 t_1 + \beta_2 t_2;$$
$$= t_1 + t_2 + \frac{s - \beta_1 t_1 - \beta_2 t_2}{\beta_3}, \quad s > \beta_1 t_1 + \beta_2 t_2.$$

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