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ESTIMATE OF THE AVERAGE DURATION OF THE OVARIAN FOLLICLE GROWTH

A COMPARATIVE STUDY IN TWO STRAINS OF RABBIT

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INTRODUCTION

There exists no accurate informations on the time of the growth and the renewal of growing follicles in the rabbit ovary (Hill & White 1933).

In the adult rabbit, it has been shown that the levels of gonadotropins vary little with the time (Blanc, Hulot 1982). Tnis is quite different in the sheep since during the oestrous cycle of the ewes, the levels of FSH vary considerably according to a waveform pattern (Lalhou-Kassi et al., 1983). It is known, furthermore, that the mitotic activiy of granulosa cells is temporarily increased after repeated injections of FSH.

It can therefore reasonably be thought that in the rabbit, the values of the parameters characterizing the proliferative activity of follicular cells will vary little during a short period of time, and may be accurately estimated.

So we estimated the time of the growth of the follicle in adult 20 weeks old rabbits of two strains differing for their natural ovulation rate by using the metaphase arrest technique (Wright & Appleton 1980). If we suppose there are few important modifications of the cell-cycle parameters during the experiment, it will be possible to estimate simply the doubling time of granulosa cells.

MATERIAL AND METHODS

The colchicine, the vincristine or their derivatives, when they are injected in vivo or in a cell culture, increase the percentage of cells which get blocked in methaphase (Tannock 1965). Consequently it is possible estimate indirectly through this method the doubling time of cell population; it has been shown indeed (Puck & Steffen 1963) that when the age distribution of the cell population is a negative exponential, the proportion of blocked metaphases increases linearly with the time of application of the blocking agent. The doubling time of the cell population is connected to the slope of the linear regression by the following relation $\ln (1 + M(t)) = 0,693 (t+tm)/Td$, where ln (.) is a neperian logarithm and $0,693 = \ln 2$, M (t) is the mitotic index of the cell population and is expressed as a percentage, M (t) is estimated on different times after the injection of the blocking agent, tm is the duration of the mitosis and Td is the doubling time of the cell population (Wright and Appleton 1980).

A single injection of 1,30 mg/kg(b.w.) colchicine was given to each rabbit intravenously. Adult nulliparous 20 weeks old rabbits of the New Zealand and Californian strains weighing about 3 kg were used for the experiment.

The adult rabbits of the Californian strain ovulate naturally 2 eggs more than those of the néozealand strain (Hulot, Mariana 1985).

24 females in each strain were alloted randomly within 6 lots; each lot was associated to a given interval between the time of the colchicine injection and the time of fixation of the ovary for each female. The corresponding intervals were 0, 1h, 2h, 3h...4h, 5h. Only one ovary for each female was recovered. Normal follicles were classified in 13 classes of increasing size between 100 m and 800 m. The lower and upper limits of the size classes were distributed according to a geometric progression of rate 1,19; we took account of the degenerating metaphases to estimate the mitotic index (Wright and Appleton 1980).

A pycnotic index of metaphase degeneracy was expressed as a proportion of degenerating metaphases. The effects of the genotype of the females, the sizes of the follicles, the time of colchicine application on the pycnotic index were tested through a variance analysis. We also tested the validity of the Puck and Steffen's model, the homogeneity of the slopes of the regression line for the two strains through a univariate variance analysis (Dagnélie 1970).

Nevertheless the natural approach is a multivariate one since each feamle is described by a vector associated to the 13 mitotic indexes in the 13 classes of size; it enables a simultaneous estimate of the parameters of the linear regression in each class of follicle and of the correlation coefficients between the parameters of the different classes of follicles. Since these estimates are non equal to zero, the parameters are consequently not independant.

The accuracy of the total time estimate of the follicle growth is also calculated. It is possible to deduce the mean doubling time of granulosa cells of follicles with a given size through the formula: b= 0,693/Td.

With b : the slope of the straight line of regression in a given size class between the mitotic index of granulosa cells and the time of application of the colchicine.

Td : The corresponding doubling time of granulosa cells in hours. At last, the time Tfi of transit of a follicle through a class of size (i) is estimated. The follicle enters the class with Ni granulosa cells and leaves it with Ni+1 (Pedersen 1969) Tfi = Td/0,693 x ln Ni+1/Ni.

The total numbers of cells Ni, Ni+1 associated to each one of the 13 classes of size are indicated in table (1).

RESULTS

The mitotic index of the granulosa cells increases significantly with the time of application of the colchicine (fig. 1. 2A. 2B), in each class of size and for the 2 strains of rabbits; the departure from linearity is not significant in any class and for either strain; the departure from parallelism between the two lines of regression associated to each strain for a given class of follicle is not significant.

The slopes of the regression lines increase, become maximum in class of follicles the size of which lies between 476 and 576 m (class 9); we deduce that the doubling time of granulosa cells decreases as the size of the follicle increases, it becomes minimum and increases again.

There exists significant correlations between the slopes of the regression lines associated to consecutive classes of size: the values of the correlation coefficients are almost all positive.

The time between the colchicine injection and the collection of the ovary, the size of the follicle, the strain of the female modify significantly the pycnotic index (fig. 3); the pycnotic index increases with the time of application of the colchicine, and the size of the follicle, and it is on average more important in the Neozealand strain than in the Californian one.

The growth curve of a follicle is not different for the two strains since there exist no differences between the slopes of the lines used to calculate the doubling times Td in each class of size.

On the beginning (fig. 4) the growth starts slowly: 90% of the entire time of growth is devoted to the transit in the first class of size; the growth becomes accelerated in the 6th class of size; the number of granulosa cells in each follicle becomes multiplied by 18 in 9 days between the 7th and the 13th class of size; later the growth becomes slower.

DISCUSSION

After having validated the blocked-metaphase technique, we estimated the average time of the growth of a follicle in the rabbits of two strains with different ovulation rates; it takes on average 97 days for the full growth of a follicle the initial size of which is 100 m.

The time of the growth is the same in the 2 strains. It is an average value; it does not take account the many possible regulations of intra or axtraovarian origin. There exists indeed relations between the rate of growth of follicles belonging to a class of size and the number of follicles in the class; Banoin, Mariana 1987 show indeed that in the ewe the proliferation of granulosa cells measured by the mitotic index in the follicles larger than 1,2 mm was negatively correlated with the number of follicles on the estrus period.

We can notice that the number of follicles is more important in the first classes of size where the growth is slower ; 80% of the total time of the growth of follicle is spent to grow from 100 m to 200 m; this result could suggest that steadily new primordial follicles les than 100 m start growing.

In fact, it is not the case since we observe very few mitosis in follicles less than 100 m in size.

Another possibility is that in the early life of the female, many little follicles less than 100 m start their growth and build a reserve of follicles the size of which is larger than 100 m and less than about 200 m in size (Faddy et al., 1976).

This built up reserve decreases slowly during the life course of the female (Read et al., 1979) either by atresia, or by growth towards larger follicles and preovulatory follicles lastly.

There exists a renewal of the whole population of growing follicles from the reserve during the life of the female but it decreases with the age.

REFERENCES

BANOIN M., MARIANA J.C., 1987. Personnal communication.

BLANC M., HULOT F., 1982. Secrétion des hormones gonadotropes au cours de la puberté chez des lapines de race californienne et néozélandaise. 3ème Journée de Recherches Cunicole en France. ITAVI ed Paris Communication 12.

DAGNELIE P., 1970. Théorie et Méthodes statistiques. ed DUCULOT S.A. Gemblou.

FADDY M.J., JONES E.C., EDWARDS R.G., 1976. An analytical model for ovarian follicle dynamics. The J. of Experimental Zoology 197. 2 173-185.

HILL M., WHITE W.E., 1933. The growth and regression of follicles in the oestrous rabbit. J. Physiol. 80 174-178.

HULOT M., MARIANA J.C., 1985. Effet du génotype, de l'âge et de la saison sur les follicules préovulatoires de la lapine 8 heures après la saillie. Reprod. Nutr. Develop. 25 (1A) 17 32.

LAHLOU KASSI A., SCHAMS D. & GLATZEL P., 1983. Plasma gonadotrophin concentrations during the oestrous cycle and after ovariectomy in two breeds of sheep with low and high fecundity. J. Reprod. Fert. 70 165-173.

PUCK T.T. & STEFFEN J., 1963. Life cycle analysis of mammalian cells. I a method for localizing metabolic events within the life cycle and its application to the action of colcemide and sublethal doses of X irradiation. Biophysical Journal 3 379 397.

READ K.L.Q., MARIANA J.C., DE REVIERS M.M., 1979. Some statistical models for ovarian folliculogenesis in infant rats. Ann. Biol. Anim. Bioch. Biophys. 19 1419-1433.

TANNOCK I.F., 1965. A comparison of the relative efficiencies of various metaphase arrest agents. Experimental Research 47 345 356.

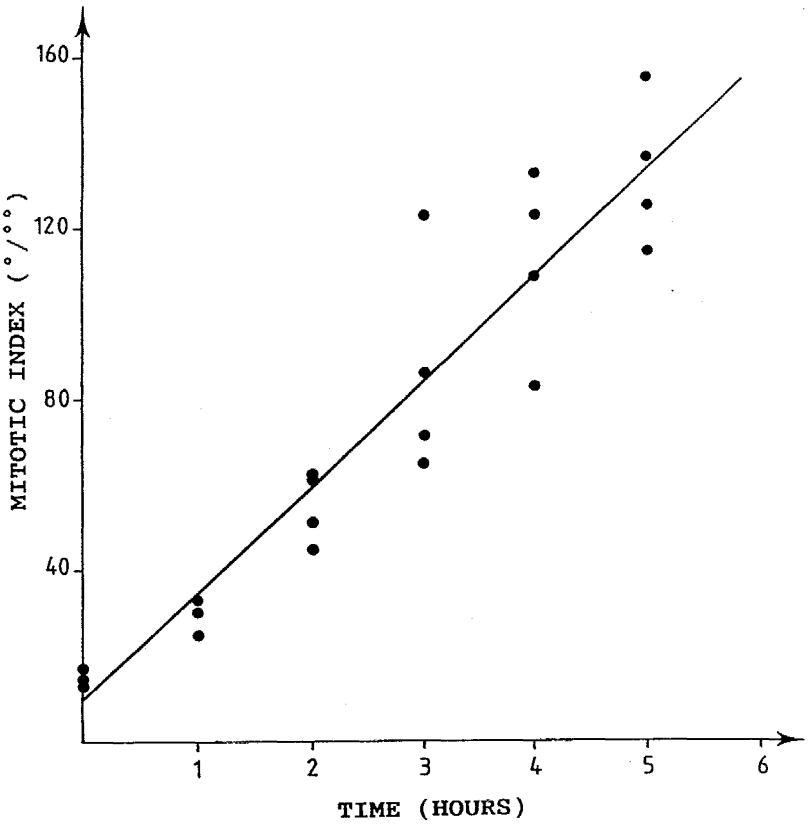
WRIGHT N.A. & APPLETON D.R., 1980. The metaphase arrest-technique. A critical review. Cell tissue Kinet 13 643 663.

Class of size	Diameter (m)	Granulosa cells number Ni - Ni+1	length of growth TFi (days)	cum ulative length
1	100,0-119,0	724-1155	35,45 (11,6)*	35,45 (11,6)**
2	119,0-141,0	1155-1843	26,74 (17,7)	62,19 (22,2)
3	141,0-168,0	18432940	14,3 (3)	76,55 (23,03)
4	168,0-200,0	2940-4689	6,7(1,2)	83,26 (23,4)
5	200,0-237,8	4689-7479	2,84 (0,3)	86,10 (23,5)
6	237,8-282,8	7479-11886	1,33 (0,14)	87,43 (23,5)
7	282,8-336,3	11886-19032	1,02 (0,10)	88,46 (23,6)
8	336,3-400,0	19032-30359	0,89 (0,07)	89,34 (23,6)
9	400,0-475,6	30359-48430	0,81 (0,06)	90,15 (23,6)
10	475,6-565,6	48430-77258	0,82 (0,06)	90,98 (23,6)
11	565,6-672,7	77258-123248	1,13 (0,07)	92,10 (23,6)
12	672,7-800,0	123248-196617	1,75 (0,21)	93,85 (23,6)
13	800 >	196617 >	3,52 (0,61)	97,37 (24)

Table 1 Values of the estimates of the different parameters for each class of size.

* Standard deviation of the length of the growth in each class (i).
** Standard deviation of the cumulative length of the growth.

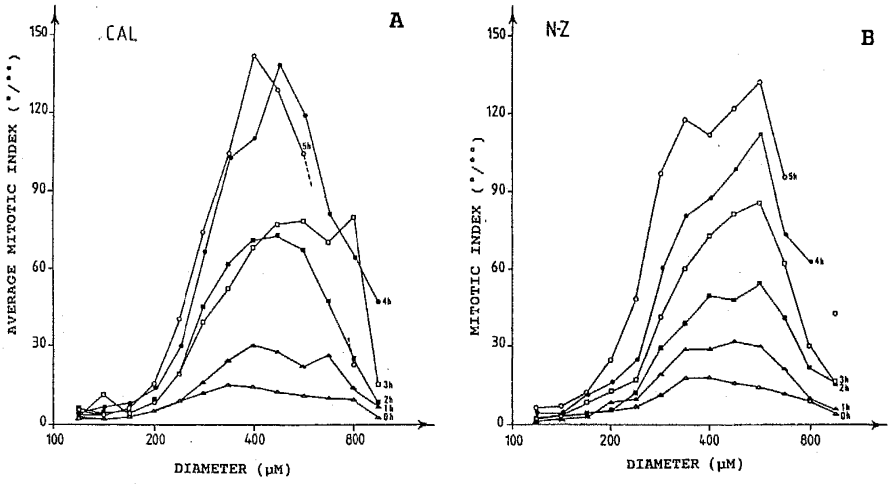
FIG.1.



THE ACCUMULATION OF GRANULOSA CELLS METAPHASES WITH TIME.

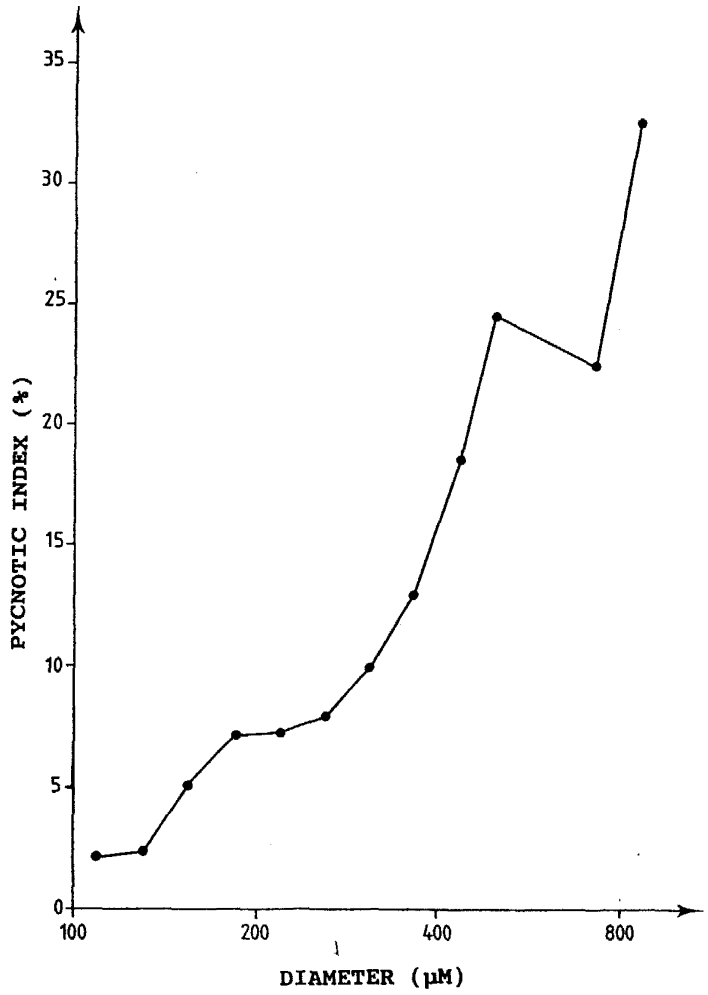
* **ABSCISSA** : time interval between administration of colchicine and fixation of the ovary in hours.
* **ORDINATE** : mitotic index of granulosa cells (/).
(Each point represents the average value of the mitotic index of follicles in the 10th class of size for each the 4 NZ females).

FIG.2.(A-B)



MITOTIC ACTIVITY OF THE GRANULOSA CELLS DURING THE GROWTH OF THE FOLLICLE AFTER DIFFERENT TIME INTERVALS BETWEEN ADMINISTRATION OF COLCHICINE AND FIXATION OF THE OVARY IN HOURS (0, 1H, 2H, 3H, 4H, 5H).

FIG.3.

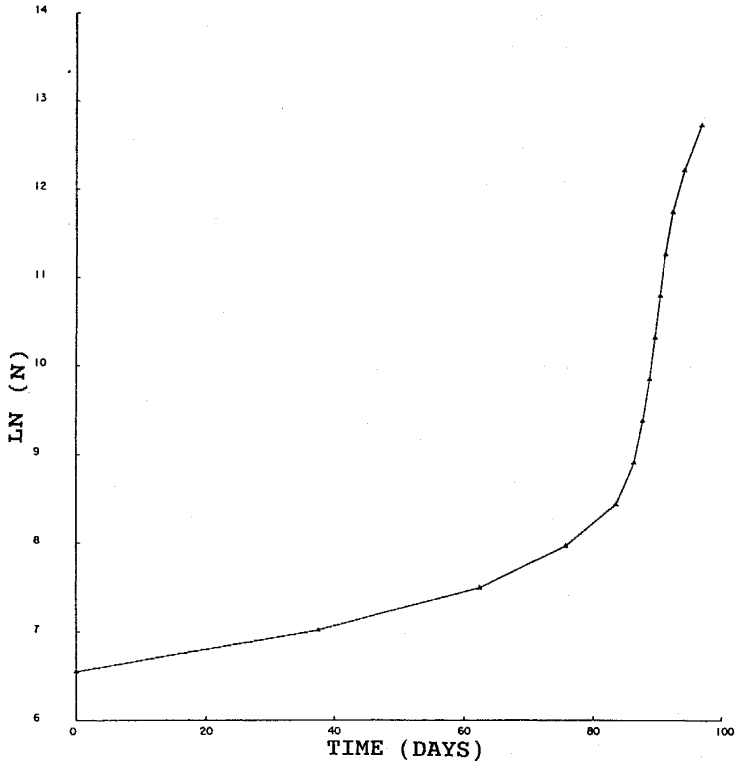


DEGENERATIVE LOSS OF METAPHASES BLOCKED BY COLCHICINE

ACCORDING TO THE DIAMETER OF THE FOLLICLE.

* **ABSCISSA** : diameter of follicles on a logscale.
* **ORDINATE** : pycnotic index of granulosa cells expressed as the percent of degenerated metaphases to the total number of metaphases.

FIG.4.



RELATION BETWEEN THE LOGARITHM OF THE NUMBER OF CELLS (N) IN A WHOLE FOLLICLE AND THE TIME IT TAKES TO GROW.

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RESUME

ESTIMATION DE LA DUREE DE CROISSANCE D'UN FOLLICULE OVARIEN. ETUDE COMPARATIVE DANS DEUX SOUCHES DE LAPIN.

MARIANA J.C., HULOT F., TOMASSONE R., DERVIN C., POUJARDIEU B.

La durée moyenne estimée de la croissance d'un follicule d'ovaire de lapin, de la taille de 100 m (follicule à 2 couches de cellules folliculeuses) à la taille de 800 m (follicule préovulatoire) est de 97 jours (sd : 24 jours).

Elle est la même pour les 2 souches de lapin étudiées : californienne et néerlandaise dont les taux moyens d'ovulation maternelle diffèrent de 2 unités.

La méthode des métaphases bloquées a été utilisée pour estimer la durée de croissance des follicules dans chacun des 13 stades qui on été définis entre 100 et 800 m.

On a estimé les valeurs de l'index mitotique des cellules de la granulosa après différentes durées d'application de la colchicine : 0, 1h, 2h, 3h, 4H, 5h, la dégérescence des métaphases a été prise en compte dans cette estimation ; on a montré que, pour chacun des 13 stades considérés, ces valeurs augmentent de façon linéaire en fonction de la durée d'application de la colchicine, cette augmentation linéaire n'est pas différente pour les 2 souches - la durée de cropissance dans chaque stade est inversement proportionnelle à la pente de la droite.

Chaque femelle (48) est décrite par un vecteur observation déféni par les index des 13 classes. Le modèle statistique est alors un modèle multivariable, il permet de faire une estimation simultanée des paramètres de la régression linéaire pour chaque classe. Cette analyse permet d'estimer les coefficients de corrélation entre les paramètres des classes ; ces estimations ne sont pas nulles : les paramètres estimés ne sont pas indépendants. Les estimations des coefficients de corrélation ont été utilisées pour estimer correctement la précision de la durée totale.

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SUMMARY

ESTIMATE OF THE AVERAGE DURATION OF THE OVARIAN FOLLICLE GROWTH. A COMPARATIVE STUDY OF TWO STRAINS OF RABBIT.

MARIANA J.C., HULOT F., TOMASSONE R., DERVIN C. POUJARDIEU B.

The average estimate of the duration of the growth of a follicle in the rabbit ovary, having an initial size of 100 m (two layers of follicular cells) and a preovulatory size of approximately 800 m, is 97 days (s.d. : 24 days). It is the same one in the two strains of rabbit: the californan and the new zealand one, whose natural ovulation numbers differ by two ovulations.

The metaphase arrest technique with colchicine was used to estimate the duration of the growth of the follicles in the 13 classes of size which were defined between 100 m and 800 m. The values of the mitotic index of the granulosa cells were estimated after different blocking times by colchicine: 0 hr, 2 hr, 3 hr, 4 hr, 5 hr. We take account of the metaphase degenerations in the estimates.

In each stage of growth, the adjusted values of the mitotic index increase linearly with the time between the colchicine injection and the collection of the ovaries for the histology. The linear increase is the same for the two strains of rabbit, and the doubling time of the granulosa cells is conversely proportional to the slope of the adjusted straight line.

Each of the 48 females of the experiment is described by one observation vector defined by the indexes of the 13 classes of size. The statistical model is therefore a multivariate one; it allows a simultaneous estimate of the parameters of the linear regression in each class of size.

The analysis allows us to estimate the coefficients of correlation between the class parameters; these estimates are different from zero; therefore the estimated parameters are not independent. The estimates of the correlation coefficients were used to estimate accurrately the total time of the growth of a follicle.