

Effect of the stage of the oestrous cycle on the follicular population in pony mares

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Summary. In the middle of the breeding season, 16 pony mares ($n = 4$ per day) were slaughtered on four different days (days 6, 14, 17 and the preovulatory day) of the oestrous cycle, day 0 being the day of the last ovulation. All the ovaries were examined histologically; the number, size and atresia (defined by granulosa cell pyknosis) of all follicles larger than 1 mm in diameter were studied, using a Kryptome-video recorder-TV system.

Follicular distribution of all the sizes studied (1-5 mm, 5-10 mm, > 10 mm in diameter) was very similar in the right and left ovaries. However, compared to the other ovary, the ovary with a luteal structure had fewer ($P < 0.05$) healthy follicles larger than 10 mm.

All follicles larger than 10 mm in diameter at day 6 were atretic. At day 14, a group of normal follicles larger than 10 mm was found in all the animals, and number of follicles larger than 10 mm was significantly increased ($P < 0.01$). The largest normal follicle has increased in size at day 17 compared to day 14. However, a significant increase in the number of follicles undergoing early atresia was observed ($P < 0.05$). Except for the large preovulatory follicle, all the follicles larger than 10 mm were atretic just before ovulation.

There was a relationship between the follicular population and the duration of the follicular phase; the number of normal follicles 1-10 mm in diameter and the duration of the follicular phase were highly correlated ($R = -0.56$; $P < 0.02$).

Introduction.

Variation in the length of natural or induced follicular phases is a key problem in the management of the mare; season has been said to be responsible for some of this variation (Palmer, 1978), as the follicular phases early in the breeding season are longer and more variable than those in the middle of the breeding season. But at all times of the year, there are substantial differences between individual mares. These differences seem related to follicular function since the lengths of the intervals between luteolysis and ovulation are correlated with the concentrations of total plasma oestrogens at the time of luteolysis (Palmer, 1978).

However, our knowledge of follicular development in this species is limited

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since observations have been restricted to postmortem macroscopic examination (Warsawsky *et al.*, 1972) and to study of the final maturation of the preovulatory follicle by rectal palpation (Nishikawa, 1959), echographic examination (Palmer and Driancourt, 1980) and assay of oestradiol-17 β (Oxender *et al.*, 1977) or total oestrogens (Palmer and Terqui, 1977). The mechanism of the selection of the follicle that will ovulate is still unknown.

In the present study, the number of follicles and their size distribution were determined at various times during the oestrous cycle of the mare in order to determine : 1) if there was a relationship between the relative distributions of these parameters in both the ovaries of individual animals ; 2) if changes occurred during the oestrous cycle and 3) whether between-animal differences in follicular distribution could be related to variations in the length of the follicular phase.

Material and methods.

Twenty pony mares, 3 to 6 years old and weighing 230 to 310 kg, were put under natural light variations and fed dried lucern, corn and oats in amounts adjusted to maintain body weight.

In the middle of the physiological breeding period (July), oestrus was induced by injecting prostaglandin F_{2 α} analogue. The mares were checked daily for oestrous behaviour, and follicular changes were assessed by daily rectal palpation. Four mares in poor physical condition failed to ovulate and were eliminated from the study. On the day of ovulation, the remaining 16 were divided randomly into four equal groups. Three groups (groups 1, 2 and 3) were slaughtered on days 6, 13 and 17, respectively, after ovulation ; the animals of group 4 were slaughtered on a day (days 17, 18, 20 and 21, respectively) when an large, soft, preovulatory follicle was found by rectal palpation.

The ovaries were prepared and the follicles were assessed, as described previously (Driancourt *et al.*, 1981). Immediately after slaughter, the ovaries were fixed with cryoprotection, quickly frozen in dry ice and stored at - 20 °C. They were then cut into 250- μ m thick sections using a cryostat, and an image of the cut surface was recorded on videotape after each cut. The largest profile of all the follicles larger than 1 mm in diameter was readily identified and measured. The presence or absence of atresia was determined by applying standard histological methods to fragments of individual follicles taken from the frozen sections. A follicle was defined as being in early atresia when the only sign of deterioration in the section studied was the presence of more than five pyknotic nuclei. Advanced atresia was defined by the disorganization of the granulosa layer, and late atresia by the absence of granulosa cells and the collapse of the antrum. Owing to the fact that follicles in late atresia have no physiological significance, they were not included here. The population of growing follicles in each ovary was divided into classes numbered from 1 to 6, having corresponding diameters of 1-5 mm, 6-10 mm, 11-15 mm, 16-20 mm, 21-25 mm and > 25 mm, respectively.

The onset of the follicular phase was assumed to be synchronous with luteolysis which was defined as the time when the progesterone levels, measured

by the method of Palmer and Jousset (1975), decline to 1 ng/ml. The end of this phase, *i.e.* the time of ovulation, was determined by rectal palpation.

Statistical methods. — The following non-parametric tests (Siegel, 1956) were used: the Wilcoxon signed ranks test for paired data (differences between ovaries with or without luteal structure and between right and left ovaries); the one-way analysis of variance of Kruskal and Wallis for distribution differences at different stages of the cycle; the Spearman rank correlation coefficient to determine the relationship, on the one hand, between the number of follicles in the various follicular groups and, on the other, between the number of follicles and the length of the follicular phase. Appropriate corrections were made for any ties that occurred. Owing to the low number of follicles in classes 4, 5 and 6, these classes were pooled for statistical analysis.

Results.

Right vs left ovary. — There was no significant difference between the right and the left ovary as to the total number of follicles (normal and atretic) or the number of normal follicles only. The same tests carried out on data on the individual classes 1-5 mm, 6-10 mm and > 10 mm did not show any differences between the right and the left ovaries either.

Effect of luteal structures. — The Wilcoxon signed ranks test, used to compare an ovary with a luteal structure (*corpus luteum* or *corpus albicans*) to contralateral-one, again showed no differences in the total number of follicles or in the number of normal follicles. The same test applied to data on the individual classes 1-5 mm and 5-10 mm did not show any difference associated with luteal structure either. However, the ovary with the luteal structure had fewer ($P < 0.05$) normal follicles larger than 10 mm and tended to have a lower total number ($0.05 < P < 0.1$) of normal and atretic follicles of that size (fig. 1). With this one exception, both ovaries in all animals were very similar, so the data of the two ovaries were pooled.

Mean follicular distribution per animal. — A schematic presentation of the total follicular population is shown in figure 2. The mean total number of follicles > 1 mm was 25, 8 of which (32 p. 100) were classed as normal and 17 (68 p. 100) as atretic. Two (12 p. 100) of the atretic follicles were undergoing early atresia and 15 (88 p. 100) advanced atresia.

However, marked individual variations occurred between animals, and the total follicular population varied between 8 and 34 follicles per animal (fig. 3). It is noteworthy that the extent of atresia was quite similar in mares with high and low pooled follicular populations; mares ($n = 7$) possessing between 10 and 20 follicles exhibited 64 p. 100 atretic follicles, while those ($n = 9$) with between 20 and 31 follicles had 62 p. 100 atretic follicles.

An examination of the relationships between the number of follicles in the different size groups shows that significant correlations link the follicular populations of classes 1 and 2 ($r = 0.89$; $P < 0.01$), classes 1 and 3 ($r = 0.49$; $P < 0.05$) and classes 2 and 3 ($r = 0.61$; $P < 0.01$). However, no correlation

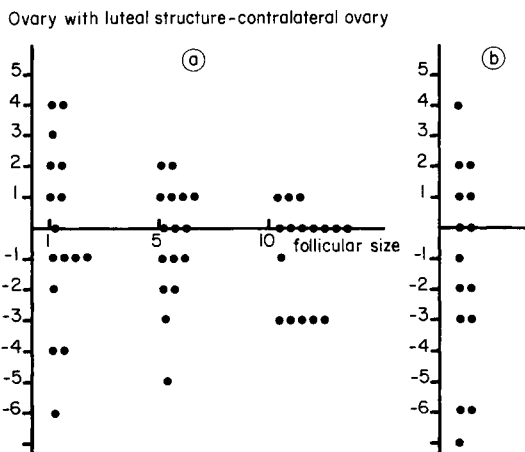


FIG. 1. — Differences (ovary with corpus luteum or corpus albicans vs contralateral ovary) in (a) the number of follicle (normal + atretic) per size class or (b) the total number of follicle. Each dot is an animal having k' follicles more or less in the ovary with a luteal structure than in the contralateral ovary. k' varies on the y-axis.

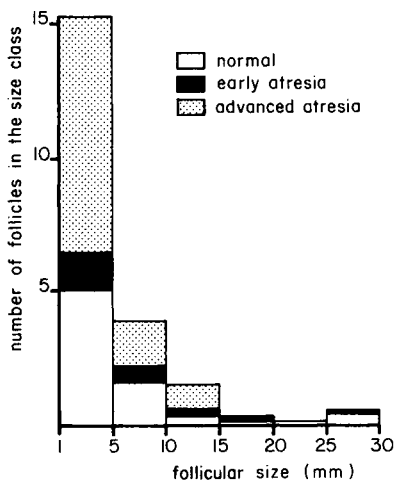


FIG. 2.

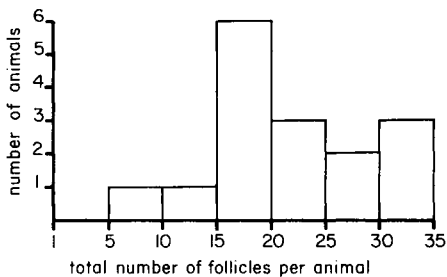


FIG. 3.

FIG. 2. — Mean follicular population per animal.

FIG. 3. — Individual variations in the follicular populations per animal.

was found between the small-follicle groups (1, 2, 3) and the large-follicle groups (4 + 5 + 6) ($r = 0.05$ between 1 and 4 + 5 + 6 ; $r = 0.03$ between 2 and 4 + 5 + 6 ; $r = 0.24$ between 3 and 4 + 5 + 6).

It appears that the size of the follicular population was related to the duration of the follicular phase since significant correlations were found between the number of normal follicles 1 to 10 mm in diameter and the duration of the

follicular phase preceding slaughter ($r = -0.56$; $P < 0.025$), and between the number of normal follicles 5 to 10 mm in diameter and the duration of the previous follicular phase ($r = -0.52$; $P < 0.05$) (fig. 4). Thus, for these size classes, the duration of the follicular phase was inversely proportional to the size of the follicular population. Apart from this exception, the ovaries of mares having short and long follicular phases were indistinguishable.

Effect of oestrous cycle stage on follicular kinetics. — The variations in the total number of follicles and in the number of follicles in the various size classes in the 16 mares studied reveal that individual differences are so great that they overshadow those between days (table 1). However, when considering the normal follicles, a clearer pattern is distinguished (table 2).

At postovulatory day 6, no normal follicle was larger than 10 mm in diameter. The diameters of the largest normal follicles of the animals in this group were 9,

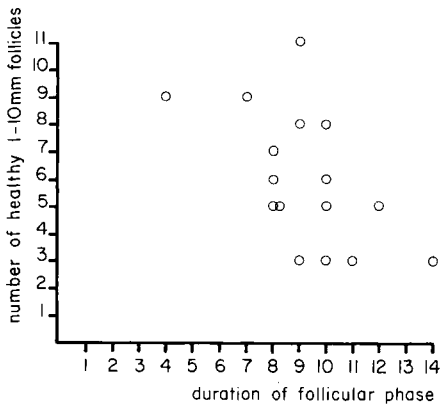


FIG. 4.

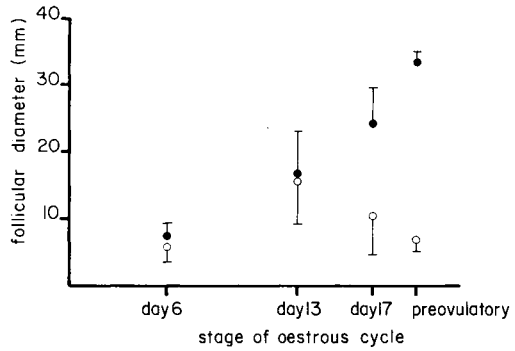


FIG. 5.

FIG. 4. — Relationship between the number of healthy 1-10 mm follicles and the duration of follicular phase.

FIG. 5. — Effect of the stage of the oestrous cycle on the diameters of the two largest healthy follicles.

TABLE 1
Mean total number of follicles per animal

Stage of cycle	Follicular size (mm)					
	1-5	5-10	10-15	15-20	20-25	> 25
Day 6	18 ± 5.6	4.3 ± 3.6	0.75 ± 1.5	0	0.25 ± 0.5	0
Day 14	15 ± 4.8	3.5 ± 2.4	1.75 ± 1.0	0.25 ± 0.5	0.25 ± 0.5	0.25 ± 0.5
Day 17	13 ± 5.1	2.3 ± 2.6	0.75 ± 1.0	0.75 ± 0.5	0.75 ± 0.5	0.75 ± 1.0
Preovulatory	15 ± 3.3	5.7 ± 4.2	2.75 ± 1.0	0.25 ± 0.5	0.25 ± 0.5	1
Mean	15.2 ± 4.7	3.9 ± 3.2	1.5 ± 1.3	0.31 ± 0.5	0.13 ± 0.5	0.5 ± 0.6

TABLE 2
Number of normal follicles per animal per size class
 (Follicles undergoing early atresia are shown in brackets)

Stage of cycle	Follicular size (mm)																		
	1-5				5-10				10-15				15-25				+ 25		
Day 6	4. (0)	2. (1)	7. (1)	3. (0)	1. (0)	1. (0)	2. (0)	6. (0)	0. (0)	0. (0)	0. (0)	0. (0)	0. (0)	0. (0)	0. (0)	0. (0)	0. (0)	0. (0)	0. (0)
Day 14	5. (1)	9. (0)	8. (0)	2. (0)	2. (0)	2. (0)	0. (0)	1. (0)	2. (0)	1. (0)	2. (0)	0. (0)	0. (0)	0. (0)	1. (0)	0. (0)	0. (0)	0. (0)	1. (0)
Day 17	7. (0)	3. (1)	5. (5)	5. (3)	2. (3)	0. (0)	0. (0)	3. (1)	0. (2)	0. (0)	0. (0)	0. (1)	1. (0)	0. (0)	1. (0)	1. (1)	1. (0)	0. (0)	0. (0)
Preovulatory	3. (3)	5. (4)	6. (1)	5. (2)	1. (1)	3. (3)	0. (0)	2. (1)	0. (2)	0. (0)	0. (0)	0. (0)	0. (0)	0. (0)	0. (0)	1. (0)	1. (0)	1. (0)	1. (0)

7, 5 and 8 mm, respectively. Nevertheless, some larger atretic follicles were present at this stage ; the diameters of the largest of these follicles were 21, 10, 9 and 8 mm, respectively.

Each of the day-14 animals had a group of normal follicles larger than 10 mm. The number and size of such follicles were, respectively, 2 of 11 mm, 1 of 11 mm, 2 of 14 and 12 mm each and 2 of 26 and 25 mm each. The increase in the number of normal follicles larger than 10 mm was significant ($P < 0.01$), and it induced an increase in the mean diameter of the first and second largest follicles (fig. 5). Furthermore, compared to day 6, the extent of atresia at this stage was reduced in follicles 1 to 10 mm in diameter (61 vs 71 p. 100) and significantly depressed in larger follicles ($P < 0.02$) (30 vs 100 p. 100) (tables 1 and 2). It is noteworthy that while follicular status was quite homogeneous between animals at day 6, there were marked dissimilarities at day 14 : one animal already had large follicles, while only small (10-15 mm) follicles were found in the others.

Compared to day 14, the size of the largest normal follicles at day 17 had increased further, individual values reaching 27, 30, 20 and 22 mm. But, compared to day 14, an increase in the percentage of atresia was also evident at this stage in follicles larger than 10 mm in diameter (58 vs 30 p. 100 ; $P < 0.05$) : the second largest follicle was in early atresia in two of the four animals (fig. 5).

Except for the large preovulatory follicles (33, 32, 36 and 35 mm in diameter, respectively), at the preovulatory stage there was no non-atretic follicle larger than 10 mm. The sizes of the second largest healthy follicles in the four animals were 7, 8, 5 and 7 mm in diameter, respectively ; there was 70 p. 100 of atresia in follicles between 1 and 10 mm in diameter and 76 p. 100 in larger follicles (tables 1 and 2).

Discussion.

Several studies on follicular populations in the mare have only considered follicular number and size (Pineda and Ginther, 1972 ; Warsawsky *et al.*, 1972).

However, to obtain a true picture of follicular growth and regression, information on atresia is needed. Most of the large-follicle population is atretic in the mare as in the ewe (Brand and De Jong, 1973 ; Cahill, Mariana and Mauléon, 1979) and the cow (Mariana and Nguyen Huy, 1973).

The technique used in this study provides information on the size, number and atresia of all follicles larger than 1 mm in diameter. This seems adequate for studying ovarian activity throughout the oestrous cycle. First, atresia is quite rare until the follicles reach 1 mm in diameter, although it is noticeable as soon as they reach 350 μm in diameter (Driancourt, 1979) ; secondly, the preovulatory follicle probably emerges from follicles of this size range.

An examination of inter- and intraovarian differences leads to the following conclusions. Although the left ovary has been frequently reported to ovulate slightly more often than the right (Osborne, 1966 ; Ginther, 1979), the two ovaries are actually very alike in this respect. Similar findings have been reported in sheep for small preantral follicles as well as for large antral ones (Cahill *et al.*, 1979 ; Kassi-Lalhou and Mariana, unpublished).

Contrary to the results of Warsawsky *et al.* (1972), we found differences between the ovary with a luteal structure (*corpus luteum* or *corpus albicans*) and the contralateral ovary. As ovulation occurs at random in either ovary (Driancourt and Palmer, unpublished), this fact is intriguing.

Intraovarian relationships are evident, if the correlations linking the number of follicles in the various size groups are considered. Between classes 3 and 4, there is a clear break in the interregulation of the number of follicles present in the different size classes. The follicular numbers in classes 1, 2 and 3 are significantly linked, while the numbers in the larger classes, unaffected by differences in the small-follicle population, may be regulated by hormonal events.

Despite the fact that different animals were used to study the follicular population at various stages of the cycle, we propose the following working hypothesis for follicular kinetics throughout the oestrous cycle. Follicular growth occurs continuously during the oestrous cycle but during the early luteal phase that growth is quite slow and the follicles never exceed 10 mm in diameter without becoming atretic. A stimulation between postovulatory days 6 and 14, that is in the middle to late luteal phase, induced some follicles to increase to more than 10 mm in diameter. Thereafter, the progressive extension of atresia results in the individualization of the preovulatory follicle.

This hypothesis and our histological results are supported by other results obtained using different techniques. Before ovulation, ablation of the ovary with the large follicle results in an ablation-ovulation interval of 13.5 days ($n = 4$) (Driancourt, unpublished observations). Thus, with the exception of the large preovulatory follicle, there is no follicle at the preovulatory stage immediately competent to mature, confirming that all the other follicles are atretic. This situation is very close to what has been observed in the monkey (Goodman *et al.*, 1977) and quite different from that found in the ewe (Land, 1973).

Furthermore, injection of a prostaglandin (PG) at postovulatory day 6 results in an 11-day interval between treatment and ovulation ($n = 15$), with no animal showing a short interval (Driancourt and Palmer, unpublished). This lack of a

rapid response to PG treatment also lends support to the idea that atresia is widespread in all large follicles during the early luteal phase.

The stimulation of follicular activity at the end of luteal phase and the increase of atresia thereafter have already been described (Ginther, 1979), but further investigation is needed to understand the mechanisms of this stimulation.

Thus, continuous follicular growth occurs in the pony mare in average physical condition in the middle of the breeding season, and one hormonally privileged period is evident towards the end of luteal phase, as in cows (Marion and Gier, 1971 ; Mariana and Nguyen Huy, 1973), ewes (Turnbull, Braden and Mattner, 1977 ; Cahill *et al.*, 1979) and women (Gougeon, 1979). A similar conclusion was reached by Wesson and Ginther (1981) after studying a large number of ovaries from the slaughterhouse. These results show that waves of follicular growth do not occur in pony mares. Peak plasma oestradiol concentrations, which have been claimed to occur during the luteal phase (Van Rensburg and Van Niekerk, 1968), are rare in pony mares : only about 20 p. 100 of the animals exhibit such peaks (Driancourt and Palmer, unpublished results).

Our experiments have tried to determine the relationships between the variation of the duration of the follicular phase and the follicular population ; a highly significant correlation was found between the pooled number of normal 1-10 mm follicles or the number of normal 5-10 mm follicles and this duration. This correlation may be explained in one of the two ways : 1) the follicles in larger populations are more sensitive to gonadotrophic stimuli, or 2) the growth rate is directly proportional to the size of the follicular population. At the present time, no data supporting the second hypothesis are available. Furthermore, examination of the plasma oestrogen levels of individual animals (Palmer and Terqui, 1977) does not provide any evidence of differences in the rate of terminal follicular growth.

Thus, variation in the duration of the follicular phase is partly due to the number of follicles available. Nevertheless, the factors explaining 70 p. 100 of the variability, and not linked to follicular populations, need further research.

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Résumé. Pendant le milieu de la saison sexuelle, 16 ponettes ont été abattues à 4 stades du cycle (4 animaux par stade) : j6, j14 et j17 post-ovulation et au stade préovulatoire. L'examen histologique des ovaires a été effectué par une technique à congélation couplée à un système magnétoscope-télévision et donne des informations sur le nombre, la taille et l'atrésie — définie par la présence de pycnose dans la granulosa — de tous les follicules de diamètre supérieur à 1 mm.

Aucune différence entre l'ovaire droit et l'ovaire gauche n'a été trouvée, ceci quelle que soit la classe de taille considérée (1-5 mm, 5-10 mm, plus de 10 mm). Par contre l'ovaire portant le corps jaune présente significativement ($P < 0,05$) moins de follicules sains de taille supérieure à 10 mm que l'autre ovaire.

A j6, il n'existe pas de follicule sain de taille supérieure à 10 mm. A j14, par contre, chaque animal présente au moins un follicule sain de diamètre supérieur à 10 mm et l'augmentation du nombre de tels follicules est significative ($P < 0,01$). A j17, on note une augmentation du diamètre du plus gros follicule sain, ainsi qu'une augmentation signifi-

tive du nombre de follicules en début d'atresie ($P < 0,05$). Au stade préovulatoire à l'exception du gros follicule préovulatoire, il n'existe pas de follicule sain d'un diamètre supérieur à 10 mm.

Une liaison existe entre les effectifs folliculaires et la durée de la phase folliculaire : le nombre de follicules sains d'une taille comprise entre 1 et 10 mm de diamètre est corrélé significativement ($r = -0,56$; $P < 0,02$) avec la durée de celle-ci.

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