Reprod. Nutr. Dévelop., 1983, **23** (2 A), 207-215.

Seasonal effects on ovarian follicular development in pony mares

M.-A. DRIANCOURT, A. PRUNIER, E. PALMER, J.-C. MARIANA

Station de Physiologie de la Reproduction, I.N.R.A. Nouzilly, 37380 Monnaie, France

Summary. To define ovarian follicular kinetics in the equine ovary during anestrus and the breeding season, the follicular population of pony mares was investigated at mid-anestrus and at the beginning and end of the breeding season. There was a clear effect of season on the exit of reserve (primordial and initiated) follicles since at the beginning of the breeding season we noticed a higher mitotic index for the smaller preantral follicles, leading to an accumulation of small and medium antral follicles. In contrast, the ovaries sampled during anestrus or at the end of the breeding season were very similar ; only preovulatory development was lacking in anestrous ovaries. However, atresia was unaffected by season.

Introduction.

Horses in temperate latitudes are seasonal breeders and ovulation usually occurs during the long natural photoperiod (Van Niekerk, 1967 ; Ginther, 1974, 1979) thus defining a winter and an early spring anestrus followed by a late spring and summer breeding season. However, most horse breeders want their mares to be covered during the winter and early spring when the ovulatory rate is low (0 to 10 % in ponies, 20 to 50 % in large mares) (Palmer, 1978 ; Ginther, 1979). Induction of ovulation is therefore needed but the kinetics of follicular growth in the ovaries during anestrus are unknown.

Furthermore, seasonal variations in gonadotrophin levels are well documented in ovariectomized (Garcia and Ginther, 1976 ; Freedman, Garcia and Ginther, 1979) as well as intact (Turner, Garcia and Ginther, 1979) mares. For example, in castrated mares, FSH has been shown to be lower during anestrus than during cyclicity (Freedman, Garcia and Ginther, 1979), while in intact mares, FSH concentrations follow a bimodal profile during the estrous cycle in the early breeding season and a unimodal profile later in the season (Ginther, 1979 ; Turner, Garcia and Ginther, 1979).

The present study was undertaken (1) to describe ovarian follicular populations and kinetics during anestrus and (2) to determine if follicular populations vary throughout the breeding season and if this variation is associated with known fluctuations of FSH levels.

Material and methods.

Fifteen Welsh pony mares were used in this study. They were 2 years old, in good physical condition, weighed around 230 kg and had all exhibited at least one cycle during the previous year.

In December 1979, while they were all in anestrus, the mares were randomly allocated to three groups (n = 5 per group).

— *Group I :* hemicastrated during anestrus. This physiological status was checked by bi-weekly blood sampling to assay progesterone according to Terqui and Thimoner (1974). Surgery was performed around February 1st, a period when these mares were judged to be in deep anestrus due to : (1) lack of progesterone for at least 2 months, (2) the fact that the size of the largest follicle did not exceed 15 mm, as shown by rectal palpation, and (3) previous data (Palmer, personal communication) showing that the end of anestrus occurs around May 8th in such animals.

— *Group II :* hemicastrated at the preovulatory stage during the early breeding season. Cyclicity was detected by bi-weekly progesterone assays (Terqui and Thimonier, 1974). When all 5 mares had exhibited at least one cycle, daily estrus detection was performed. As soon as a mare was in estrus, its follicular development was followed until the preovulatory stage (*i.e.* soft follicle exceeding 35 mm in diameter), using echography and rectal palpation (Palmer and Driancourt, 1980). The mean day of hemicastration was May 15th.

— *Group III :* hemicastrated at the preovulatory stage during the second part of the breeding season. We carried out a procedure similar to that in group II mares, starting on August 1st, the mean day of surgery being around August 15th.

As both ovaries have been shown to present very close populations (Driancourt, Mariana and Palmer, 1982), one ovary of anestrous animals and the ovary bearing the large follicle of cyclic mares were removed, fixed in Bouin Hollande solution and cut into serial sections 10-µm thick. One out of five sections was mounted, stained with hematoxylin and examined microscopically.

All follicles larger than 50 µm in diameter were counted, measured and then grouped into the 5 classes previously described (Driancourt *et al.,* 1982).

Furthermore, all follicles were checked for atresia ; a follicle was considered atretic when more than five pyknotic nuclei were counted on the section studied (stage I : early atresia). Two later stages were determined according to Kenney *et al.* (1979) : stage II (advanced atresia) when pyknosis is widespread and numerous granulosa cells float in the antrum and stage III (late atresia) when the granulosa is completely lacking, except in the oocyte area. The terminal stages when fibroblasts invade the follicle were not counted.

In all healthy follicles, the number of granulosa cells per follicle in the section studied was established either directly, by counting all the cells in follicles smaller than 120 µm in diameter, or indirectly ; in larger follicles, it was calculated as the product of the area the cells occupied and cellular density measured at ten different points on the follicle, using a circular graticle.

Thereafter, all cells undergoing mitosis were counted in the same section and the mitotic index was obtained by dividing the number of cells in mitosis by

the total number of granulosa cells. To study differences in the mitotic index, a weighted mean and its standard deviation were computed according to Meier (1953).

Owing to the small number of animals, non-parametric tests (Siegel, 1956) were used : the Kruskal and Wallis one-way analysis of variance and the Mann-Whitney U-test to compare follicular populations among groups. To determine differences in the mitotic index, we used an analysis of variance after angular transformation of the percentages.

Results.

Cyclic ovarian activity. — As expected, none of the 5 mares in group I showed cyclic ovarian activity at the time of surgery, while 5/5 mares in group II and 4/5 mares in group III were cyclic. In all group II and III ponies, the ovulation rate was 1.

Follicular populations. — Using the Kruskal and Wallis one-way analysis of variance to compare the number of follicles per class among groups (fig. 1), we did not find any significant differences when comparing the numbers of initiated and preantral follicles at the three physiological statuses studied. The trend to higher numbers of follicles in these classes during the early breeding season was due to an animal with an extremely numerous population (table 1). In contrast, significant seasonal differences were detected in small and medium antral follicles. Concerning small antral follicles, significantly more follicles were present during the early breeding season compared to anestrus ($U = 3$; $p < 0.05$) or late breeding season ($U = 0$; $p < 0.01$). However, the numbers of such follicles in the ovaries of anestrous or late breeding season animals were very similar. A like trend existed in medium antral follicles. Significantly more follicles of this size were found in ovaries collected during the early breeding season than at anestrus ($U = 2$; $p < 0.01$) and the end of the breeding season

TABLE 1

Individual total numbers of follicles per size class. Each line is one animal

	Initiated	Preantral	Small antral	Medium antral	Large antral
Anestrus	15	6	7	12	5
	*21	2	3	5	1
	78	15	8	8	0
	61	3	1	2	1
	30	9	1	4	2
Beginning of breeding season	57	14	9	15	2
	23	9	10	20	1
	55	9	7	11	2
	226	28	7	40	1
	10	4	10	10	3
End of breeding season	59	3	2	6	1
	35	5	5	3	1
	21	9	5	5	2
	39	8	3	11	1
	52	6	4	9	3

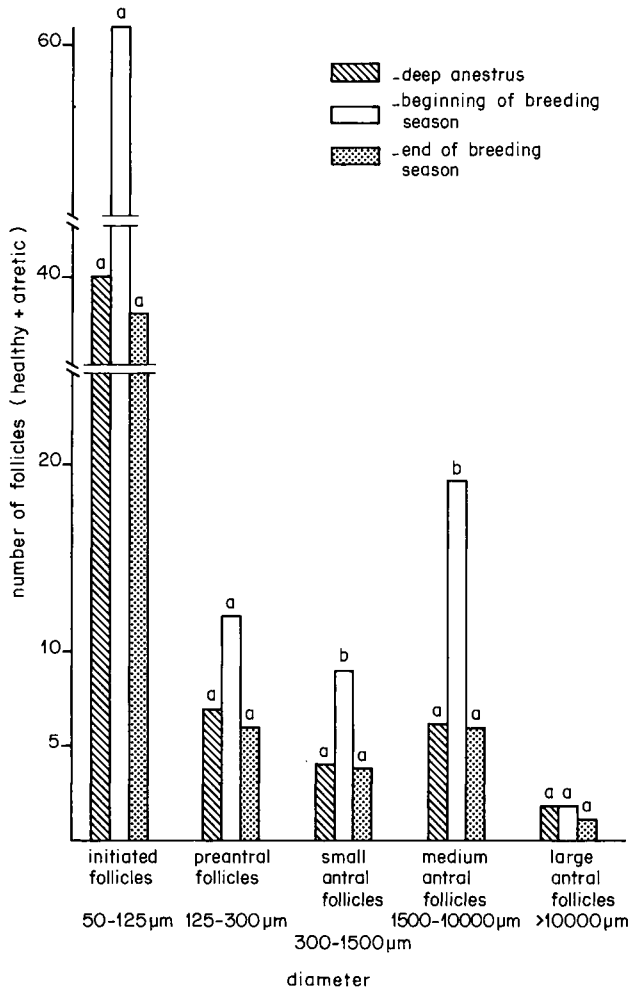

FIG. 1. — *Mean total* (healthy and atretic) *number of follicles per size class at the three physiological (statuses) studied.*

(U = 1.5 ; p < 0.01). But the population of medium antral follicles was equal between group I and III mares. Surprisingly, the population of large antral follicles was very similar among the three groups.

Atresia. — With our criteria, atresia was only observed in antral follicles. Since there was very little atresia in small antral follicles and it was poorly estimated in large antral follicles, due to the low number of available follicles, only data concerning medium antral follicles are presented (fig. 2). As the percentage of atresia and the relative percentages of early and advanced atretic follicles among the groups were similar, a higher number of atretic follicles was noticed in the early breeding season compared to anestrus (U = 0 ; p < 0.01)

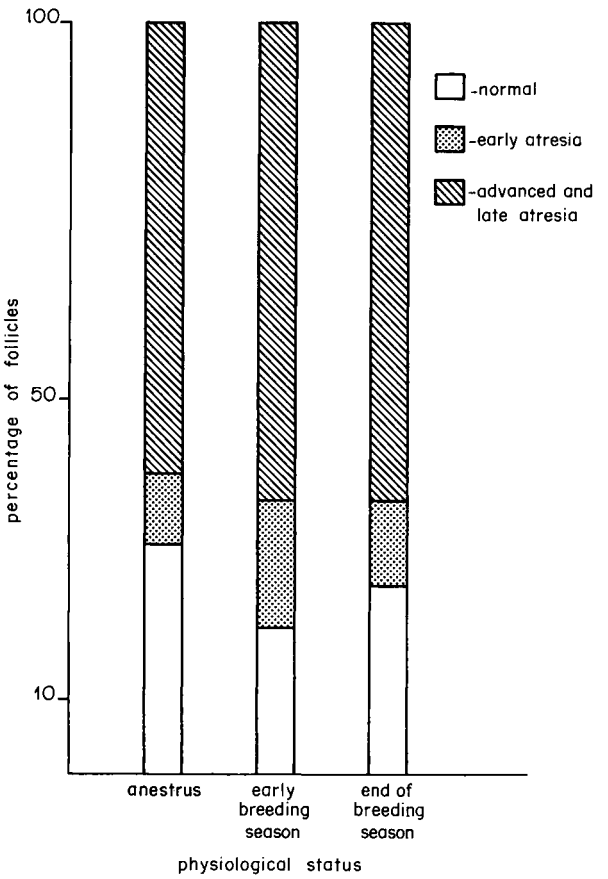

FIG. 2. — *Relative extent of healthy, early atretic, and advanced atretic follicles according to physiological status.*

and the end of the breeding season ($U = 1$; $p < 0.01$), due to the higher number of follicles in this season.

Mitotic index. — The mean mitotic index and its standard deviation were calculated for the initiated, preantral and healthy small antral follicles in each group (fig. 3). This parameter was not measured in larger follicles owing to the small number of such healthy follicles.

There were no significant differences among any of the groups as to the mean mitotic index of initiated follicles. But unlike initiated follicles, smaller preantral follicles (*i.e.* between 120 and 190 μm in diameter) showed a significant increase ($F = 5.14$; $p < 0.05$) in mitotic index during the early breeding season compared to the other two groups. The proliferative activity of granulosa cells in larger follicles among the groups was similar with very high individual differences.

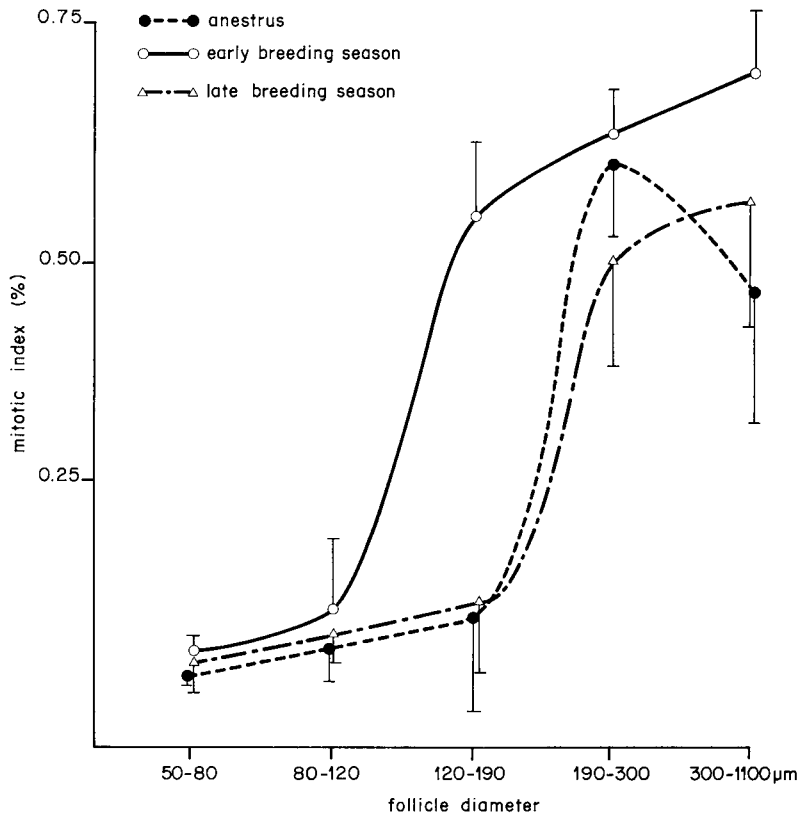

Fig. 3. — *Mean* (± SD) *mitotic index value of various size classes in the three experimental groups.*

Discussion.

Pony mares were used in this study because the reproductive season is well delineated into anovulatory and ovulatory seasons : (1) almost 100 % of pony mares exhibit anestrus during winter compared to around 50 % in large mares (Palmer, 1978 ; Ginther, 1979) and (2) a persistent corpus luteum occurs very seldom in ponies. Furthermore, young animals (*i.e.* less than 2 years old) still show a clearer pattern of seasonality because all those in our herd are non-cycling during winter (Palmer, personal communication), although a similar trend has not been demonstrated in a slaughterhouse study (Wesson and Ginther, 1981). Thus, young pony mares are a valuable model for the study of seasonal effects on follicular development in the equine.

While the first two experimental groups were well defined, we have no data concerning the day of the last ovulation after hemicastration in animals from the third group. However, two points suggest that they were close to entering anestrus : (1) one of the 5 animals was not cycling and (2) results from Wesson and

Ginther (1981) show that the percentage of ovulatory young pony mares is around 60 % in September and 20 % in October.

The present study provides new information on follicular populations throughout the year since, in previous studies, only palpable follicles (Turner, Garcia and Ginther, 1979) or those observed on slices of postmortem ovaries (Wesson and Ginther, 1981) have been considered (*i.e.* follicles over 2 mm in diameter).

The important features of the present study are firstly, the close populations of ovaries sampled during anestrus and at the end of the breeding season and, secondly, the peculiar kinetics and populations noticed during the beginning of the breeding season compared to the other two stages studied.

Whatever the size class studied, follicular numbers were similar in group I and III ovaries, the only noticeable difference being the lack of follicles larger than 15 mm in diameter during anestrus. Thus, as in ewes (Turnbull *et al.*, 1978 ; Cahill and Mauléon, 1980), the only part of folliculogenesis lacking during winter is preovulatory development. Furthermore, anestrous follicles seem to be sensitive to endogenous or exogenous hormones since Oxender, Noden and Hafs (1977) have noticed some spikes of estradiol-17β, and ovulation has been successfully induced with pituitary extracts in a fortnight in seasonally anovulatory mares (Douglas, Nuti and Ginther, 1974 ; Lapin and Ginther, 1977). Another conclusion that can be drawn from a comparison of group I and III ovaries is the fact that follicular populations less than 10 mm in diameter are relatively independent of the hormonal milieu. Although hormonal assays were not done on our animals, differences in estrogens, progesterone, LH and possibly FSH between anestrus and the end of the breeding season are clear (Ginther, 1979). They do not seem to result in differences in follicular populations. Also worthy of note are the equal numbers of initiated follicles at these two physiological statuses. Such follicles have previously been shown to belong to the reserve of small follicles (Driancourt *et al.*, 1982). Thus, in contrast to what has been proposed in the ewe, there is no restocking of small reserve follicles during anestrus (Cahill and Mauléon, 1980), such follicles being used during the breeding season. This is further supported by data involving light treatments (Kooistra and Ginther, 1975) which show that hastening the onset of the breeding season by light does not induce it to end earlier and that continuation of maximal photoperiod during summer and fall prolongs the period of cyclicity.

Follicular development seems to be original in the early breeding season compared to the other stages studied. At this period, a higher exit of the reserve of small follicles (primordial and initiated) occurs, as demonstrated by the higher mitotic index of the smaller preantral follicles and by the accumulation of small and medium antral follicles. At the moment, the mechanisms inducing this higher exit are unclear.

The similar extent of atresia among the three stages studied is intriguing as FSH levels are markedly different among these stages and as FSH is a key hormone in the control of atresia (Zeleznik, 1981 ; Di Zerega *et al.*, 1981). However, it must be kept in mind that at the preovulatory stage, there is considerable atresia compared to the end of luteal phase and the beginning of estrus (Driancourt, Mariana and Palmer, 1982). In contrast, the close values among the three groups

of the mitotic index of antral follicles were expected since a similar fact has been demonstrated in ewes (Turnbull *et al.*, 1978 ; Cahill and Mauléon, 1980).

Thus, except for the modulation of the exit of reserve follicles and the lack of very large preovulatory follicles during anestrus, season does not have much effect on folliculogenesis in the equine.

Reçu en juillet 1982.
Accepté en octobre 1982.

Résumé. *Influence de la saison sur la population de follicules ovariens chez la ponette.*

Afin de caractériser le fonctionnement de l'ovaire équin au cours de l'anœstrus et au cours de la saison sexuelle, les populations folliculaires d'ovaires d'animaux en anœstrus, début de saison sexuelle et fin de saison sexuelle ont été comptées, l'importance de l'atrésie et les valeurs de l'index mitotique calculées. La saison exerce un effet sur la sortie de la réserve de petits follicules (primordiaux et initiés). En effet, en début de saison sexuelle, l'index mitotique des plus petits follicules préantraux est significativement supérieur à celui des autres stades, ce qui induit une augmentation du nombre de follicules à petit et moyen antrum. En revanche, les ovaires prélevés en anœstrus ou fin de saison sexuelle présentent des populations très proches ; seul manque le développement préovulaire dans l'ovaire d'anœstrus. Enfin, l'atrésie ne varie pas avec la saison.

References

CAHILL L. P., MAULÉON P., 1980. Influence of season, cycle and breed on follicular growth rates in sheep. *J. Reprod. Fert.*, **58**, 321-328.

I ZEREGA G., TURNER C. K., STOUFFER R. L., ANDERSON L. D., CHANNING C. P., HODGEN G. D., 1981. Suppression of FSH-dependent folliculogenesis during the primate ovarian cycle. *J. clin. Endocr. Metab.*, **52**, 451-456.

DOUGLAS R. H., NUTI L., GINTHER O. J., 1974. Induction of ovulation and mutiple ovulation in seasonally anovulatory mares with equine pituitary fractions. *Theriogenology*, **2**, 133-142.

DRIANCOURT M. A., MARIANA J. C., PALMER E., 1982. Effect of the stage of the oestrous cycle on the follicular population in pony mares. *Reprod. Nutr. Dévelop.*, **22**, 803-812.

DRIANCOURT M. A., PARIS A., ROUX C., MARIANA J. C., PALMER E., 1982. Ovarian follicular populations in pony and saddle-type mares. *Reprod. Nutr. Dévelop.*, **22**, 1035-1048.

FREEDMAN L. J., GARCIA M. C., GINTHER O. J., 1979. Gonadotropin levels in response to season and photoperiod in ovariectomized mares. *Biol. Reprod.*, **20**, 567-574.

GARCIA M. C., GINTHER O. J., 1976. Effects of ovariectomy and season on plasma luteinizing hormones in mares. *Endocrinology*, **98**, 958-962.

GINTHER O. J., 1974. Occurrence of anestrus, estrus, diestrus and ovulation over a 12-month period in mares. *Am. J. vet. Res.*, **35**, 1173-1179.

GINTHER O. J., 1979. *Reproductive biology of the mare : basic and applied aspects.* McNaughton & Gunn Inc., Ann. Arbor., Mich.

KENNEY R. M., CONDON W., GANJAM V. K., CHANNING C. P., 1979. Morphological and biochemical correlates of equine ovarian follicles as a function of their state of viability or atresia. *J. Reprod. Fert.*, suppl. **27**, 163-171.

KOOISTRA L. H., GINTHER O. J., 1975. Effect of photoperiod on reproductive activity and hair in mares. *Am. J. vet. Res.*, **36**, 1413-1419.

LAPIN D. R., GINTHER O. J., 1977. Induction of ovulation and multiple ovulations in seasonally anovulatory and ovulatory mares with an equine pituitary extract. *J. anim. Sci.*, **44**, 834-842.

MEIER P., 1953. Variance of a weighted mean. *Biometrics,* **9,** 59-62.

OXENDER W., NODEN P., HAFS H. D., 1977. Estrus, ovulation and serum progesterone, estradiol-17β and LH concentrations in mares after an increased photoperiod during winter. *Am. J. vet. Res.,* **38,** 203-207.

PALMER E., 1978. Control of the oestrous cycle of the mare. *J. Reprod. Fert.,* **54,** 495-505.

PALMER E., DRIANCOURT M. A., 1980. Use of ultrasonic echography in equine gynecology. *Theriogenology,* **13,** 203-216.

SIEGEL S., 1956. *Non-parametric statistics for the behavioural sciences.* McGraw Hill, New York.

TERQUI M., THIMONIER J., 1974. Nouvelle méthode radioimmunologique rapide pour l'estimation du niveau de progestérone plasmatique. Application pour le diagnostic précoce de la gestation. *C.R. Acad. Sci. Paris, sér. D,* **279,** 1109-1112.

TURNBULL K. E., MATTNER P. E., GEORGE J. M., SCARAMUZZI R. J., 1978. The relation between patterns of ovarian follicle growth and ovulation rate in sheep. *Aust. J. biol. Sci.,* **31,** 649-655.

TURNER D. D., GARCIA M. C., GINTHER O. J., 1979. Follicular and gonadotropic changes throughout the year in pony mares. *Am. J. vet. Res.,* **40,** 694-700.

VAN NIEKERK C. H., 1967. Pattern of the oestrous cycle of mares. I. breeding season. *J. S. Afr. vet. med. Assoc.,* **38,** 295-298.

WESSON J. A., GINTHER O. J., 1981. Influence of season and age on reproductive activity in pony mares on the basis of a slaughterhouse survey. *J. anim. Sci.,* **52,** 119-129.

ZELEZNIK A. J., 1981. Premature elevation of systemic estradiol reduces serum levels of FSH and lengthens the follicular phase of the menstrual cycle of rhesus monkeys. *Endocrinology,* **109,** 352-355.