Actes Inst. Agron. Vet. (Maroc) 1997, Vol. 17 (4) : 245-252

Effect of breed, hemicastration and FSH on the ovarian follicular population of ewe lambs

Aziza JORIO [1¤], Jean-Claude MARIANA [2], Abdellatif LAHLOU-KASSI [3] & Mohamed HILALI [4]

(Reçu le 21/02/1997 ; Accepté le 26/06/1997)

تأثير السلالة والإخصاء النصفي والحاثة منشطة الجريب على مجموع الجريبات المبيضية عند النعاج : سلالة ولودة وأخرى قليلة النسل

باتباع طرق الدراسات النسيجية الكمية للمبيض. قارننا بين تأثير السلالة وتأثير الإخصاء النصفي و المعالجة بالحاثة منشطة الجريب بعد الإخصاء عند مجموعتين من نعاج عمرها 15 يوما تنتسبان إلى سلالتين مختلفتان من حيث معدل إباضتهما هما الدمان (وهي ولودة) وتمحضيت (وهي قليلة النسل). أظهرت نتائج هذه الدراسة أنه لايوجد فرق ملحوظ بين عدد الجريبات المبيضية وتوزيعها عند السلالتين في سن 15 يوما. كما أن الإخصاء النصفي لا يؤثر على المدى القريب على مجموع الجريبات المبيضية أو تركيز الحاثة منشطة الجريب. غير أن الإخصاء النصفي المتبوع بحقن الحاثة منشطة الجريب يؤدي عند السلالتين معا إلى ارتفاع تركيز هذه الأخيرة كما ترتفع عدد الجريبات الإبتدائية (ذات الطبقة الواحدة من الخلايا المكعبة) ويتضاعف أيضا عدد الجريبات الجوفاء. أما الجريبات الإضمحلالية فينخفض عددها إلى النصف. وتبدي السلالة الولودة (الدمان) استجابة أكثر لتأثير FSH من السلالة القليلة النسل (تمحضيت).

الكلمات المفتاحية : السلالة – نعاج – المبيض – الجريب – الإخصاء النصفي – الحاثة منشطة الجريب

Effet de la race de l'hémicastration et de la FSH sur la population folliculaire ovarienne chez les agnelles

Utilisant la méthode histologique quantitative de l'ovaire, on a comparé l'effet race, hémicastration et traitement avec FSH après hémicastration chez l'agnelle âgée de 15 jours de deux races différant par leur taux d'ovulation : D'man (haute prolificité), Timahdite (basse prolificité). Le nombre et la distribution des follicules ovariens ne diffèrent pas significativement entre les deux races à l'âge de 15 jours et l'hémicastration n'a pas d'effet à court-terme sur la population des follicles ovariens et sur la concentration de FSH. La concentration de FSH augmente après hémicastration et injection de FSH. Le nombre des follicules primordiaux (avec une couche de cellules cubiques) augmente après traitement avec FSH. Le nombre de follicules à antrum est doublé et l'atrésie est réduite de moitié chez les deux races. La race prolifique (D'man) montre une sensibilité à la stimulation par FSH plus marquée que chez la race non-prolifique (Timahdite).

Mots clés : Race - Agnelle - Prolificité - Ovaire - Follicule - Hémicastration - F SH

Effect of breed, hemicastration and FSH on the ovarian follicular population of ewe lambs

Using quantitative histological methods, we compared the ovarian effect of breed, hemicastration and FSH treatment after hemicastration in 15 days old ewe lambs of two breeds with different ovulation rates: D'man (high prolificacy), Timahdite (low prolificacy). The number and the distribution of ovarian follicles did not differ significantly between breeds at 15 days of age and the hemicastration had no short-term effect on the population of ovarian follicles and FSH concentration. The concentration of FSH increased following hemicastration and injection of FSH.The number of primordial follicles (with one layer of cubic cells) increased after FSH treatment. The number of antral follicles doubled and atresia was reduced by half in the two breeds. The prolific breed (D'man) showed a greater sensitivity to FSH stimulation than the non-prolific breed (Timahdite).

Key words : Breed - Ewe lamb - Prolificacy - Ovary - Follicle - Hemicastration - FSH

[1] Département de Biologie, E.N.S. Takaddoum, Rabat Maroc
[2] Laboratoire de Physiologie, INRA, 37380 Nouzilly, France
[3] Département fe Reproduction Animale et Insémination Artificielle, BP 6202-Instituts, 10101 Rabat, Maroc
[4] Département de Physiologie Animale, Faculté des Sciences, Fès, Maroc
¤ Corresponding author

INTRODUCTION

In earlier studies, breed was reported to be the major factor affecting the population of ovarian follicles at birth, but not at 4 weeks of age (Sonjaya & Driancourt, 1987 ; Jorio *et al.*, 1991). Also, hemicastration had no short-term effect on the number and the distribution of ovarian follicles in the cyclic heifer (Monniaux, 1982). Ovariectomy had no effect on FSH concentrations in the 15 days old ewe lamb (Foster *et al.*, 1975).

The role of Follicle Stimulating Hormone (FSH) in stimulating the growth of follicles and their protection against atresia is well documented (McNeilly *et al.*, 1991 ; Mariana *et al.*, 1991; Hay *et al.*, 1979). FSH and PMSG modify the number and the distribution of follicles according to size in the immature rat (DeReviers & Mauléon, 1973) and the cow (Monniaux, 1982). Furthermore, it has been shown that in the ewe, the response to a given dose of FSH or PMSG depends upon the prolificacy of the breed; the induced ovulation rate in immature and adult sheep is greater in prolific than in non-prolific breeds (Quirke, 1979 ; Piper *et al.*, 1982 ; Bindon *et al.*, 1986). This difference may be due to the ability of gonadotrophins to stimulate a larger number of antral!ûødlicles to reach their terminal phase of growth quicker in prolific breed than in non prolific breed. The time at wich this higher sensitivity to exogenous gonadotrophins appears has not been established.

The aim of this study was to compare the ovarian follicular population (Experiment 1) and to test the ovarian response (total number and distribution of growing and atretic follicles) to hemicastration and FSH treatment after hemicastration (Experiment 2) in 15-days old ewe lambs of a prolific (D'man) and a non-prolific breed (Timahdite) of moroccan sheep.

MATERIALS & METHODS

Experiment 1. At 15 days of age. The right ovaries of 8 D'man and 8 Timahdite ewe lambs were removed, preserved in Bouin Holland fixative and later used to estimate the number of follicles.

Experiment 2. Eight 15-day old females were used (4 D'man and 4 Timahdite). The animals were randomly allocated into 2 groups : Group 1 (2 D'man and 2 Timahdite ewe lambs) was used to test the effect of hemicastration. Group 2 (2 D'man and 2 Timahdite ewe lambs) was used to test the effect of FSH treatment.

In group 1, the animals were hemicastrated by locating and removing the right ovaries. The left ovaries were removed 3 days later.

In group 2, the right ovaries were located and removed. At the time of hemicastration and for the following 2 days, the animals received intravenously 2 mg porcine FSH (P-FSH-CY-1220/ C 100 mg Eq. Armour FSH/LH = 6.5) at 0900h. The left ovaries were collected 3 days later. The right ovary served as a control for the left ovary in the animals treated with FSH, thus allowing each animal to be its own control.

After ovariectomy, ovaries were immediately dropped into fixative (Bouin Holland), embedded in paraplast and sectionned at 5µm. Every fifth section was stained with Regaud's Hematoxilin (same as for Experiment 1).

The number and the distribution of growing and atretic follicles were analysed before and after the FSH treatment using each animal as its own control.

Blood was sampled each 30 mn for 5 hours starting 1h before hemicastration and/or first FSH injection. On the following 2 days, 2 blood samples were taken 30 mn apart just prior to the 2nd and 3rd injection.

Counting and classification of follicles. In order to avoid counting follicles more than once, the oocyte was used as a marker (Lahlou-Kassi & Mariana (1984) ; Monniaux,1982 ; Jorio *et al.*, 1991). We took into account the follicle section in which the oocyte was at its maximum size. The area and the diameter of this section was measured using a computerised planimeter (ASM Leitz). Follicles with at least 2 layers of granulosa cells were counted in Experiment 2, together with follicles with one layer of cubic cells. They were classified according to their diameter into 26 classes represented by a geometric progression of x1.19. This was the classification used by Lahlou-Kassi & Mariana (1984) and Jorio *et al.*, (1991).

A follicle was classified as normal if there was 1) no visible signs of disorganisation, 2) nor hypertrophy of the granulosa layer, 3) less than 10 pycnotic bodies and 4) no abnormal oocyte. Otherwise, it was considered atretic.

We defined the distance between the right (i) and the left (k) ovary of the same animal as :

$$d_ik = \sum_{j=1}^{26} \frac{1}{X_ij + X_kj} (X_ij - X_kj)$$

where Xij is the number of follicles of the jth class in the right ovary (i) and Xkj is the number of follicles of the jth class in the left ovay(k) (Chandon & Pinson, 1981).

The distributions of follicles by size were compared using the Kolmogorov-Smirnov test (Conover, 1980). Results were expressed as means ± s.e.m..The effects of breed (D'man and Timahdite) or of ovary (right and left) on changes in follicular population and in hormone concentration were analysed by two-way analysis of variance (Exp. 2) (Dagnelie, 1975).

The concentrations of FSH were mesured using a homologous radioimmunoassay with double antibody (Golter *et al.*, 1973). The tracer used was ovine FSH (NIADDK-OFSH) provided by Dr. Parlow. It was labelled with iodine 125 using the chloramine T method. The antibody was a rabbit antiserum, NIAMDD-anti-OFSH-1, was at final dilution of 1/112000.The non-radioactive hormone for the series of standards was ovine FSH (NIAMDD-NIH-OFSH-RP-1). The sensitivity of the assay was 0.1 ng/ml.

RESULTS

Experiment 1

The average total number of follicles (with at least two layers of cells) did not differ significantly between breeds (Normal follicles; D'man: 394.37 ± 225.39, Timahdite: 552.25 ± 326.49) (Atretic follicles; D'man: 39.37 ± 33.68,Timahdite: 58.25 ± 46.47), and neither did the distribution of follicles (Figure 1). The proportion of atretic follicles varied according to size in the two breeds.

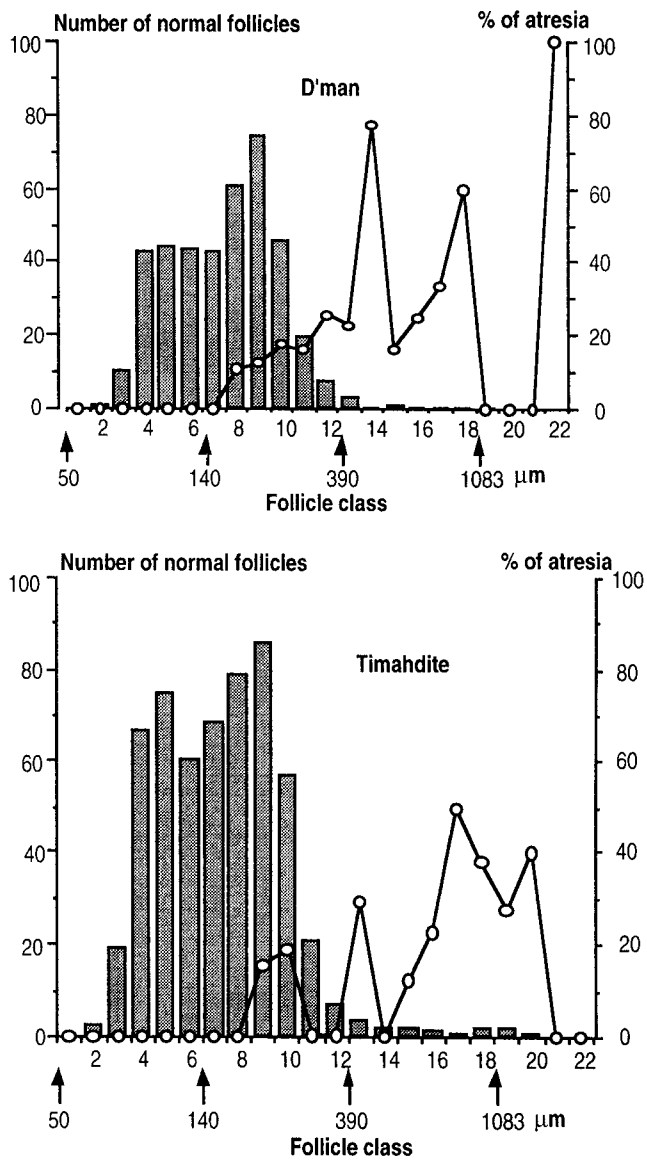

Figure 1.Distribution of follicles with 2 or more layers of cells by size (bars) and level of atresia (circles) in the ovaries of 15 days old D'man and Timahdite ewe lambs

Experiment 2

Effect of hemicastration

The number of follicles with one layer of cubic cells was not changed by hemicastration. In the D'man, the number of follicles was 288 ± 36 on the right ovary and 279 ± 12.5 on the left ovary. In the Timahdite this number of follicles was 298 ± 210 in the right ovary and 305.5 ± 231.5 on the left ovary. The distribution of follicles by size (Figure 2a) was similar between ovaries of the same animal and between breeds.

The total number of normal follicles, with at least two layers of cells per ovary, was similar, before and after hemicastration. In the D'man, the average number of normal follicles with or without an antrum was 320 ± 51 on the right ovary (RO) and 300 ± 42 on the left ovary (LO) (Figure 3a). Corresponding figures for the Timahdite were 300 ± 297 (RO) and 323 ± 299 (LO). Furthermore, a comparison between ovaries, for each class size indicates that the variation (or difference) (LO/RO x 100) in relative departure from equality (100%) is not statistically significant between the two breeds.

The number of atretic follicles did not differ between ovaries in the same animal in both breeds (Figure 3a). In the D'man, the average number of atretic follicles without an antrum was 26.5 ± 16.5

in the right ovary and 28.5 ± 20.5 in the left ovary, or with an antrum was 18.5 ± 15.5 in the right ovary and 20 ± 20 in the left ovary. In the Timahdite, no signs of atresia were seen in both ovaries.

As shown in Figure 4a, there were similar concentrations of FSH before and after hemicastration in the two breeds. In the D'man the concentration of FSH was 1.72 ± 1.64 ng/ml and 1.75 ± 1.76 ng/ml and in the Timahdite it was 0.83 ± 0.29 ng/ml and 0.83 ± 0.14 ng/ml before and after hemicatration respectively.

Effect of FSH treatment (after hemicastration)

In the D'man ewe lambs there were 544 ± 36 follicles with one layer of cubic cells in the right ovary, and 760 ± 79 in the left ovary after FSH treatment. In Timahdite, there were 516 ± 12 follicles in the right ovary and 673 ± 32 in the left ovary. After FSH treatment, there were not significant interaction between breed ovary and breed effect but there was a ovary effect ($P < 0.02$); there was a significant increase in the number of follicles with one layer of cubic cells.

The distribution of follicles size was similar for the right and left ovaries (Figure 2b). The distance between the size distribution in the 2 ovaries in the two breeds increased slightly but not significantly in favour of the D'man (1.155 and 0.995 for the D'man and Timahdite respectively).

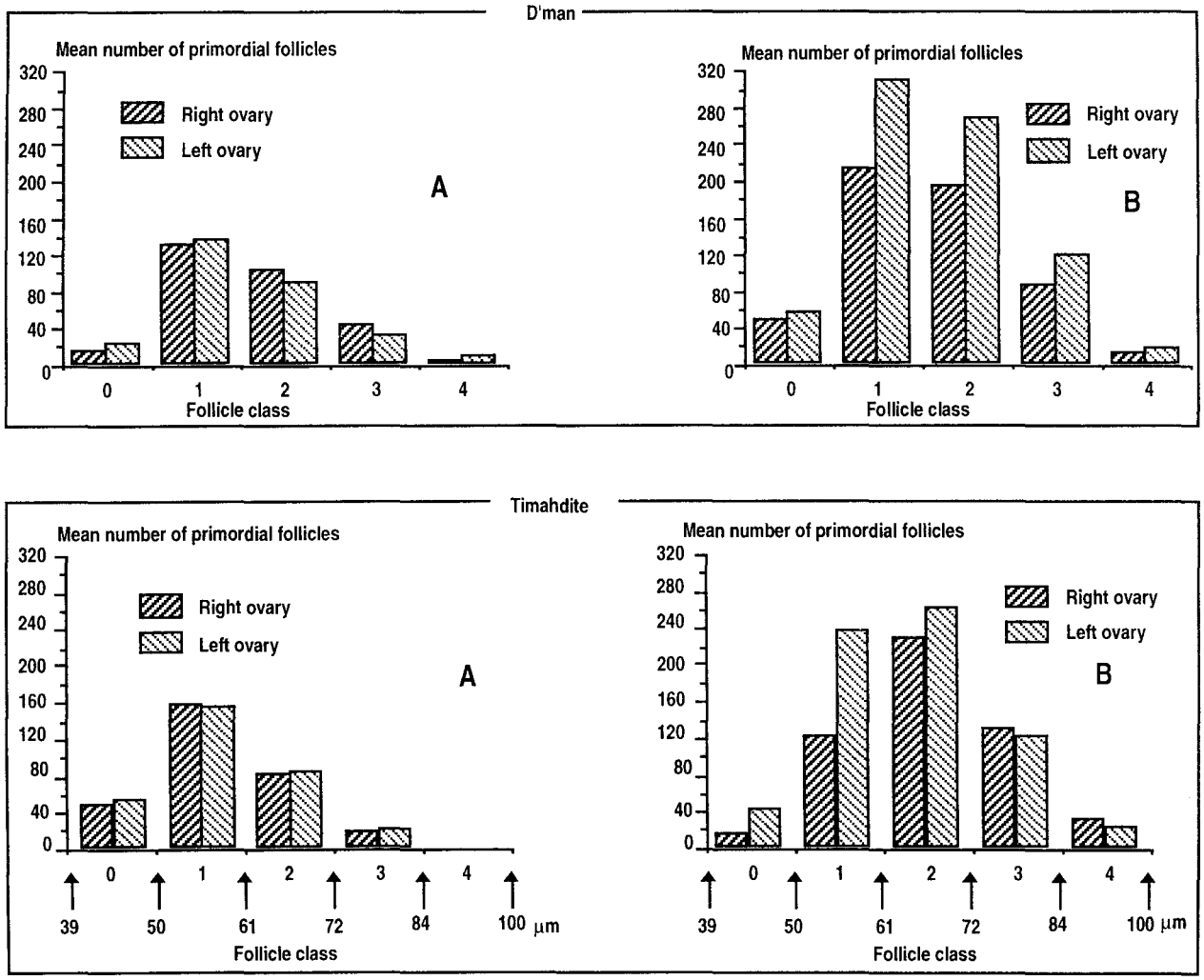

Figure 2. Distribution of primordial follicles with one layer of cubic cells by size. Effect of hemicastration (A) and FSH treatment after hemicastration (B) in D'man and Timahdite ewe lambs

There was not a breed effect, a ovary effect and a breed ovary interaction on the number of normal follicles with at least two or more layers of cells and without an antrum; it was unchanged after the FSH treatment in both breeds: D'man 486 ± 140 (RO) vs 512 ± 116.5 (LO) ; Timahdite 538.5 ± 145.5 (RO) vs 546 ± 140 (LO) (Figure 3b). There was ovary effect ($P < 0.05$) but no breed effect or breed ovary interaction on the number of normal follicles with an antrum, it was increased from 32 ± 22 to 88 ± 33 in the D'man and from 23 ± 6 to 58 ± 28 in the Timahdite ($P < 0.05$) (Figure3b). After the FSH treatment, the relative deviation (LO-RO /RO) of the mean number of normal follicles with an antrum in the control ovary and the treated ovary increased in both breeds, 175% in the D'man and 154.3% in the Timahdite.

The relative discrepancy between the right and left ovary (LO/RO x 100) started to increase from the ninth size class (234 µm) in both breeds and was maximal at the eleventh size class (320 µm).

After FSH treatment, the number of atretic follicles differ significantly ($P<0.05$) between ovaries in the same animal in both breeds, but no breed effect and breedxovary interaction (Figure 3b). The rate of atresia was reduced in all follicle classes in both breeds. The reduction in the relative deviation of the mean number of atretic follicles was greater in the Timahdite (-78% and -61.11% without and with antrum respectively) than in the D'man (-95.79% and -97.61% without and with antrum respectively) but not significantly.

In both breeds, FSH concentration were increased after the first FSH injection by about 5-folds (Figure4b) ; D'man: 1.08 ± 0.65 vs 4.63 ± 1.83 ng/ml and Timahdite 1.16 ± 0.58 vs 5.11 ± 0.16 ng/ml.

DISCUSSION

There was no significant difference between breeds in the number and the distribution of normal follicles, in the 15 days old ewe lamb, as seen by Jorio *et al.*, (1991). This is in disagreement with data observed at birth where both the number and the distribution of normal follicles were different between the 2 breeds used in this study and in other studies (Romanov and Ile-de-France) (Sonjaya & Driancourt, 1987).

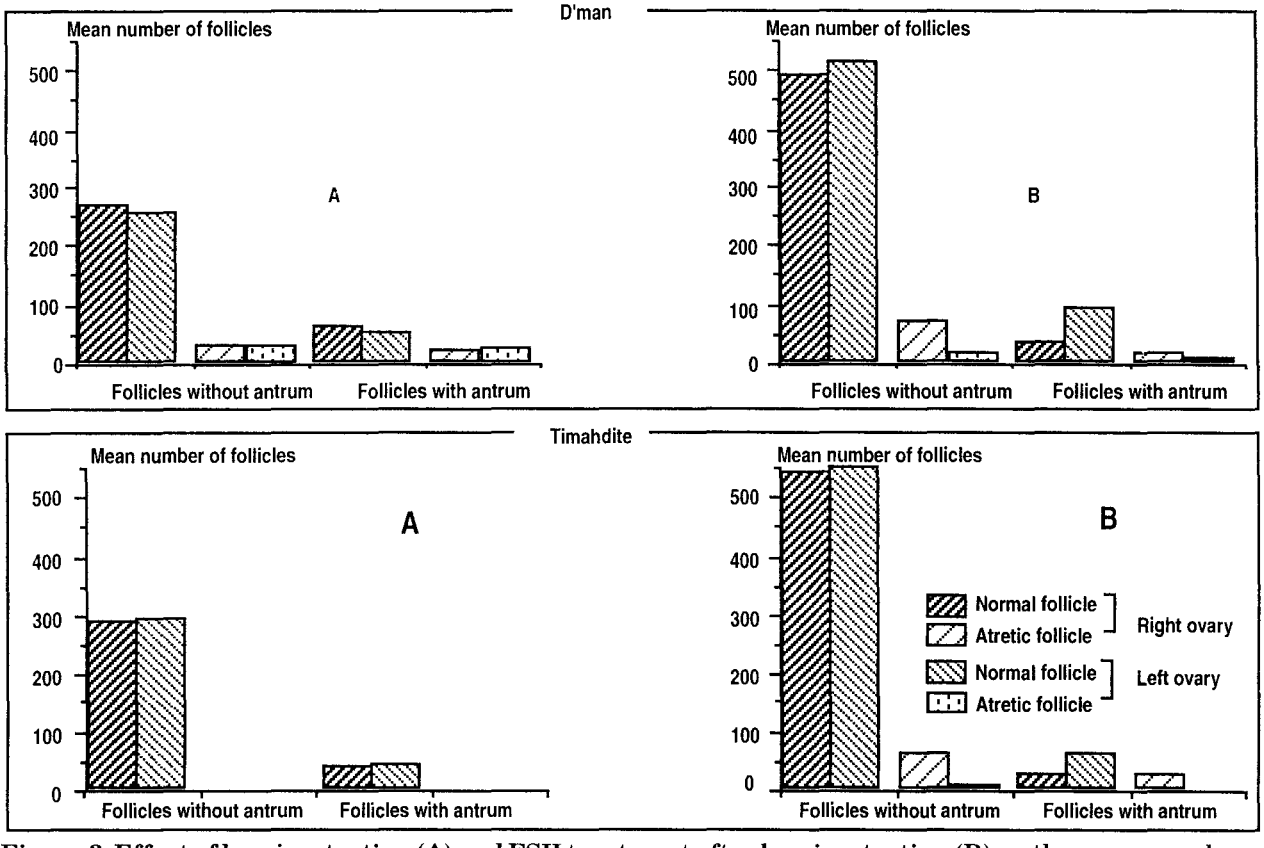

Figure 3. Effect of hemicastration (A) and FSH treatment after hemicastration (B) on the mean number of normal and atretic ovarian follicles in D'man and Timahdite ewe lambs

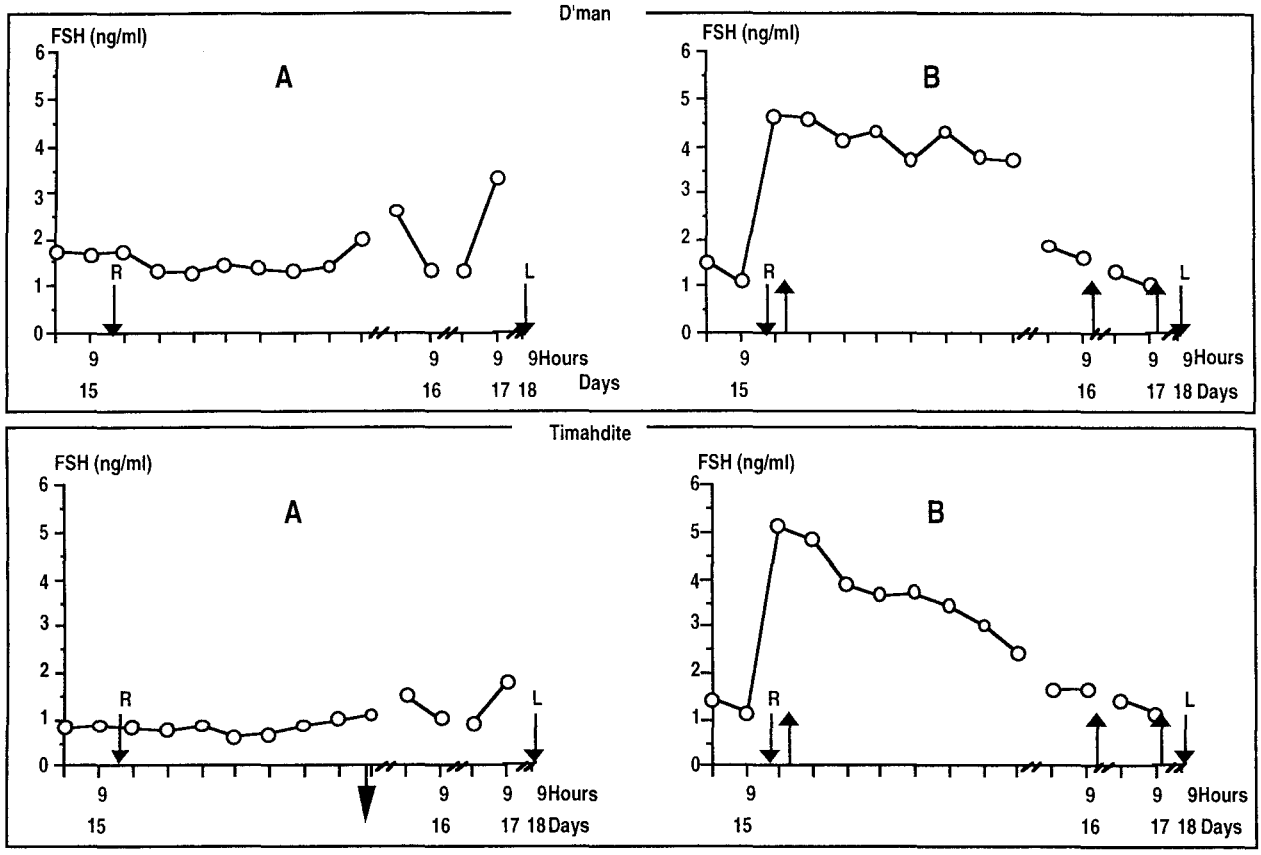

Figure 4. Effect of hemicastration (A) and FSH treatment after hemicastration (B) on plasma FSH concentrations in D'man and Timahdite ewe lambs

↓ : removal of right (R) or left (L) ovary ; ↑: injection of 2 mg P-FSH

In the15 days ewe lamb, hemicastration had no short term effect on the concentration of FSH and on the ovarian follicles with at least one layer of cells. There was no effect of ovariectomy (2-3 days) at 5 weeks of age on FSH concentration (Sonjaya & Driancourt 1989), Foster *et al.*, (1975) showed also that FSH and LH concentrations are low during 2-3 weeks after ovariectomy of 15 days old ewe lambs. This is in agreement with Monniaux (1982) who showed, in the cyclic heifer, that the distribution of growing follicles by size did not change in the 10 days following hemicastration. The compensatory increase in the number of follicles in the remaining ovary is therefore a slow process. In cyclic sheep, the increase in the number of antral follicles becomes significant 70 days after hemicastration (Dufour *et al.*, 1979). The use of the other ovary of the same animal as a control is therefore justified.

The five-fold increase of endogenous FSH after hemicastration and FSH treatment, had a net action on 3 follicle cathegories: primordial follicles (with one layer of cubic cells), antral follicles and atretic follicles. The increase in the number of follicles with one layer of cubic cells induced by exogenous FSH was similar to that observed in the prepubertal rat (de Reviers & Mauléon, 1973), in the adult rat (Mariana, 1982) and the prepubertal mouse (Lintern-Moore, 1978). This increase was due to the growth of small follicles with a diameter smaller than 40µm. Furthermore, in follicles with 1 and 2 layers of cuboidal granulosa cells,FSH-receptor was detected in ovine ovaries (Tisdall *et al.*, 1995). The number of normal follicles with an antrum increased by more than twofold after FSH treatment in both breeds. Similar results were observed in 5 and 6 week old sheep (Worthington & Kennedy, 1979) and in prepupertal rats (de Reviers & Mauléon, 1973). The dependency of antral follicles upon hormonal support was also demonstrated after hypophysectomy in the rat (de Reviers & Mauléon, 1973) and the sheep (Dufour *et al.*, 1979). In this study the total number of normal follicles without an antrum was not modified by FSH treatment but this does not necessarily mean that normal follicles without an antrum are not sensitive to FSH. They may have been growing at

a slower rate than other follicles so that changes were not easily perceptible within three days post-treatment. Another explanation may be that the transition of follicles without an antrum to antral follicles may be partly compensated for by a replenishment from the pool of small follicles. It seems from results of Experiment 2 that FSH acts by protecting follicles against atresia.

Similar results have been reported by Halpin *et al.*, (1986) who showed that gonadotrophins act on medium size follicles by reducing atresia, rather than by stimulating growth. In this study, the reduction in the number of atretic follicles with no antrum was observed in the two breeds; it was partly compensated for by an increase in the number of normal follicles without antrum, and by an increase in the number of follicles with an antrum. It seems ther that FSH stimulates the growth of such follicles and protects them against atresia. This provides further support to previous studies showing that antral follicles are stimulated to grow and are protected against atresia in many species (Braw & Tsafriri, 1980, immature rat ; Monniaux, 1982, cow ; Hay *et al.*, 1979, sheep follicles *in vitro*). The results presented herein showed that FSH acts on sheep follicles as early as 15 days of age in the two breeds studied.

The number of follicles with either one layer of cubic cells and 2 or more layers of granulosa cells were also greater in the D'man than in the Timahdite after FSH treatment. This may have been due to different numbers of follicles in the ovary at the time of FSH treatment but this is unlikely because there were no significant differences in follicle number and distribution between the control ovaries of the two breeds. However, this difference may have been due to different mechanisms associated with cell proliferation and differentiation or to differences of synthesis of FSH receptors during follicular growth.

In the Booroola Merino ovary, LH and FSH receptors appeared earlier on small follicles than in the non Booroola Merino ovary (Scaramuzzi & Radford, 1983). Follicular cells from sheep carriyng the Fec B gene (marked increases in ovulation rate) are more sensitive to gonadotrophins *in vitro* (Webb *et al.*, 1995). The responses we observed may also be due to differences in the plasma concentrations of FSH (endogenous FSH) at the time of treatment. However we observed no differences in endogeneous FSH levels among ewes of two breeds before the injection of porcine FSH. Our results which are confined to the period around 15 days of age do not give a full picture of the prepubertal pattern of FSH secretion. Other authors have observed higher levels of FSH in ewes of prolific breeds before puberty (Sonjaya & Driancourt, 1989 ; Isaacs *et al.*, 1995) and in mature ewes (Lahlou-Kassi *et al.*, 1984 ; Boulton *et al.*, 1995).

CONCLUSIONS

Results of this study showed that the number and the distribution of ovarian follicles did not differ between breeds (D'man and Timahdite) at 15 days of age. The hemicastration had no short-term effect on the population of follicles and the concentrations of FSH. The administration of 2 mg of P-FSH on the day of hemicastration and the following two days doubled the number of antral follicles, presumably through halving the rate of atresia. This effect was more marked in the prolific D'man ewe lamb than in the non-prolific Timahdite ewe lamb, thus indicating a breed-related sensitivity to gonadotrophin (FSH).

REFERENCES

Bindon B.M., Piper L.R., Cahill L.P., Driancourt M.A. & O'Shea T. (1986) Genetic and hormonal factors affecting superovulation. *Theriogenology* 25 : 53-71

Boulton M.I., Haley C.S., Springbett A.J. & Webb R. (1995) The effect of the Booroola (FecB) gene on peripheral FSH concentrations and ovulation rates during oestus, seasonal anoestrus and on FSH concentrations following ovariectomy in Scottich back face ewes. *J. Reprod. Fert.* 103 : 199-207

Braw R.H. & Tsafriri A. (1980) Effect of PMSG on follicular atresia in the immature rat ovary. *J. Reprod. Fert.* 59 : 267-272

Chandon J.L. & Pinson S. (1981) Analyse typologique: théories et applications. Ed. Masson

Conover W.J. (1980) Practical non parametric statistics. Second Edition. Ed.John Wiley & Sons

Dagnelie P. (1975) Théorie et méthodes statistiques. Vol. 2. Seconde Edition. Les Presses Agronomiques de Gembloux

Dufour J.J., Cahill L.P. & Mauléon P. (1979) Short- and long-term effects of hypophysectomy and unilateral ovariectomy on ovarian follicular populations in sheep. *J. Reprod. Fert.* 57 : 301-309

Foster D.L., Jaffe R.B. & Niswender G.D. (1975) sequential pattern of circulating LH and FSH in female sheep during the early postnatal period: effect of gonadectomy. *Endocrinologiy* 97 : 985 995

Golter T.D., Reevs J.J., O'mary C.C., Arimuz A., Schally A.V. (1973) Serum LH levels in bulls treated with synthetic luteinizing hormone-releasing hormone, follicle stimulating hormone-releasing hormone (LH-RH/FSH-RH). *J. Anim. Sci.* 37 : 123-127

Halpin D.M.G., Charlton H.M. & Faddy M.J. (1986) Effects of gonadotrophin deficiency on follicular development in hypogonadal (hpg) mice. *J. Reprod. F.* 78 : 119-125

Hay M.F., Moor R.M., Cran D.G. & Dott H.M. (1979) Regeneration of atretic ovarian follicles *in vitro*. *J. Reprod. F.* 55 : 195-207

Isaacs K.L., McNatty K.P., Condell L., Shaw L., Heath D.A., Hudson N.L., Littlejohn R.P. & Mcleod B.J. (1995) Plasma FSH, LH and inhibin concentrations in FecBB/FecBB and FecB+/FecB+ Booroola ewes and rams from birth to 12 monts of age. *J. Reprod. F.* 103 : 89-97

Jorio A., Mariana J.C. & Lahlou-Kassi A. (1991) Development of the population of ovarian follicles during the prepubertal period in D'man and Timahdite sheep. *Anim. Reprod. Sci.* 26 : 239-250

Lahlou-Kassi A., Schams D. & Gladzel, P. (1984) Plasma gonadotrophin concentrations during the oestrous cycle and after ovariectomy in two breeds of sheep with low and high fecundity. *J. Reprod. F.* 70: 165-173

Lahlou-Kassi A. & Mariana J.C. (1984) Ovarian follicular growth during the oestrus cycle in two breeds of ewes of different ovulation rate, the D'man and the Timahdite. *J. Reprod. F* 72 : 301-310

Lintern-Moore S. (1978) Initiation of follicular growth in the infant mouse ovary by exogenous gonadotrophin. *Biol. Reprod.* 17: 635-639

Mariana J.C. (1982) Compte-rendu des journées d'information ITEB-UNCEIA (Institut technique de l'élevage bovin Ed.), 53-70

Mariana J.C., Monniaux D., Driancourt M.A. & Mauléon P. (1991) Folliculogenesis. *In* : «Reproduction in Domestic Animals», Fourth Ed. Cole H.H. & Cupps P.T., Ac. Press, New york, 119-171

McNeilly A.S, Picton H.M., Campbell B.K. & Baird D.T.(1991) Gonadotrophic control of follicle growth in the ewe. *J. Reprod. Fert.* 43 : 177-186

Monniaux D. (1982) Origine de la variabilité de la réponse ovulatoire à PMSG chez la génisse. Thèse de docteur de 3ème cycle, Sciences Naturelles, Université Pierre et Marie-Curie -Paris 6-. France

Piper L.R., Bindon B.M., Curtis Y.M., Cheers M.A. & Nethery R.D. (1982) Response to PMSG in Merino and Booroola Merino crosses. *Proc. Aust. Soc. Reprod. Biol.* 14 : 82

Reviers (**De**) M.M. & Mauléon P. (1973) Effets des hormones gonadotropes sur l'ovaire de ratte immature. *Ann. Biol. Anim. Biochim. Biophys.* 13 : 177-193

Scaramuzzi R.J. & Radford H.M. (1983) Factors regulating ovulation rate in the ewe. *J. Reprod. Fert.* 69 : 353-367

Sonjaya H. & Driancourt M.A. (1987) ovarian follicles during infancy in Romanov and Ile-de-France ewe lambs. *J. Reprod. Fert.* 81 : 241-248

Sonjaya H. & Driancourt M.A. (1989) FSH concentrations and sensitivity to feed back in infant lambs from breeds differing in prolificacy. *J. Reprod. Fert.* 85 : 461-469

Tisdall D.J., Smith P., Leeuwenberg B. & McNatty K.P., Wallaceville (1995) FSH-receptor, bB inhibin subinit, follistatin, bA and a inhibin subinits and IGF-1 genes are expressed sequentially in ovine granulosa cells during early follicular development. J. *Reprod. Fert.* 15 : 12, Abstr.

Webb R., Campbell B. & Driancourt M.A. (1995) Follicular cells from sheep carrying the FecB gene are more sensitive to gonadotrophins *in vitro*. *J. Reprod. Fert.* 15 : 12, Abstr.

Worthington C.A. & Kennedy J.P. (1979) Ovarian response to exogenous hormones in six- week-old lambs. *Aust. J. Biol. Sci.* 32 : 91-99