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Summary — Follicular growth over 2 mm in diameter is mainly regulated by gonadotrophins (FSH and LH). However, it is possible that between-follicle interactions may be involved in ensuring ovulation of a specific number of follicles. The present study tested specific hypotheses regarding: (1) a stimulatory role of atretic follicles on differentiation of follicles of the next wave; (2) a stimulatory role of large follicles in *F*-gene-carrier Booroola ewes on differentiation of follicles of the same ovulatory cohort; and (3) an inhibitory role of the dominant follicle on differentiation of follicles of the same ovulatory cohort. The end points measured were aromatase activity (all trials) and granulosa cell proliferation (Booroola). The techniques used tested effects of ovarian venous serum or conditioned media or follicle co-culture. Comparison of the effects of similar protein concentrations of serum or media conditioned by healthy *versus* atretic follicles could not demonstrate a stimulatory role of the atretic follicle. This was further confirmed using ovarian venous serum draining an ovary bearing an atretic follicle. Comparison of the effects of similar protein concentration of serum or media conditioned by *F* + or ++ follicles could not demonstrate a stimulatory role of large follicles in *F* + ewes. This was supported by the observation that ovarian venous serum of *F* + or ++ ewes had similar effects on aromatase activity. Furthermore, coculture of small follicles with a large follicle of either genotype did not alter granulosa-cell proliferation compared with controls. This suggests that an alteration in the between-follicles regulation does not operate in Booroola sheep to generate their high ovulation rate. Comparison of the effects of similar protein concentrations of serum, follicular fluid from large follicles or media conditioned by large follicles showed that follicular fluid (but not conditioned media) contained an aromatase inhibitor. This suggests that dominant follicles do not secrete the aromatase inhibitor they contain.

follicle / follicular fluid / aromatase / ewe / granulosa-cell division / Booroola

Résumé — Absence d'interactions entre gros follicules dans l'ovaire de brebis. *La croissance folliculaire terminale chez la brebis est clairement régulée par les hormones gonadotropes (FSH et LH). Cependant des interactions entre follicules pourraient également jouer un rôle i) dans le renouvellement des vagues folliculaires au cours du cyclè (par un effet stimulateur du follicule atrétique), ii) dans l'édification d'une cohorte ovulatoire importante chez les femelles Booroola porteuses du gène F (par un effet stimulateur du premier follicule dominant), et iii) dans l'individualisation du follicule dominant en fin de phase folliculaire (par un effet inhibiteur de celui-ci sur les autres follicules). Les tests biologiques employés mesurent les effets de milieux conditionnés par des follicules de caractéristiques physiologiques connues ou de sérum veineux ovariens sur l'activité aromatase. La comparaison des effets de concentrations en protéines identiques de sérum ou de milieux conditionnés par des follicules sains ou atrétiques ne montre pas d'effet stimulateur des sécrétions du follicule atrétique. Ce résultat est*

confirmé par l'absence d'effet du sérum ovarien drainant un ovaire portant un follicule atrétique par rapport à l'ovaire contralatéral. Chez les brebis porteuses ou non du gène Booroola, ni des milieux conditionnés par des follicules de brebis F+, ni du sérum veineux ovarien drainant des ovaires de brebis F+ n'exercent d'effet modulateur significatif sur l'aromatase. La même conclusion est valide chez les brebis non porteuses du gène. De plus, la culture de petits follicules de chaque génotype en présence de follicules dominants de chaque génotype n'a pas permis de mettre en évidence un effet stimulateur du gros follicule sur la prolifération cellulaire de la granulosa des petits follicules. Dans les 2 tests (aromatase, prolifération cellulaire), la réponse des brebis F+ à FSH était cependant supérieure à celle des brebis non porteuses. Enfin, le follicule dominant ne sécrète pas dans le milieu d'incubation l'inhibiteur de l'aromatase qu'il contient dans le liquide folliculaire. L'ensemble de ces résultats démontre que, à partir du test aromatase, aucune interaction entre follicules n'est détectable chez la brebis.

follicule / liquide folliculaire / aromatase / brebis / prolifération cellulaire de la granulosa / Booroola

INTRODUCTION

The endocrine control of follicular growth in sheep is well documented. In the absence of gonadotrophins (hypophysectomy, GnRh agonist treatment), ovaries are devoid of follicles > 2 mm in diameter (Dufour *et al,* 1979; Driancourt *et al,* 1987; McNeilly and Fraser, 1987; McNatty *et al,* 1990). Furthermore FSH together with basal levels of LH are required to induce growth of large follicles in sheep with minimal levels of endogenous gonadotrophins (McNeilly *et al,* 1992). Manipulation of gonadotrophin levels in gonado-deprived sheep often fails to generate an ovulation rate or a number of large follicles typical (mean and variation) of the breed studied (Driancourt *et al,* 1988; Fry *et al,* 1988; Mc Neilly *et al,* 1992). This may imply that between-follicle interactions are involved in the mechanism whereby the fine-tuning of terminal follicular growth is achieved. These between-follicle regulations can, of course, be positive or negative. Indeed, a role of such regulations may explain the waves of follicular growth that occur throughout the cycle (Smeaton and Robertson, 1971; Driancourt *et al,* 1988). Renewal of waves could imply secretion of a positive regulator by the large dominant follicle as it becomes atretic. Between-follicle regulations may also explain the kinetics of growth of large follicles observed in *F* gene carriers Booroola sheep (Driancourt *et al,* 1985). This could be related to the production of a positive regulator by the first dominant follicle, which will potentialize the response of the smaller follicles to the decreasing FSH levels. The appearance of a dominant follicle may also involve such an interaction, as it could result from the production of negative regulators inhibiting growth of the other follicles as previously suggested in primates (Di Zerega *et al,* 1982).

The aims of the present study are thus to check whether positive effects of atretic follicles or negative effects of dominant follicles on differentiation of other follicles can be detected. Furthermore, we also tested whether local regulations of follicular growth were altered by the Booroola gene through the production of positive paracrine regulators by the first dominant follicle appearing.

MATERIALS AND METHODS

As between-follicle interactions can only be operative if the compounds involved are secreted, their existence was tested using: (1) media conditioned by follicles; and/or (2) ovarian venous serum. Because follicular fluid is stored inside the follicle and because proteins contained in follicular fluid rather than incubation media are not all the same on 2-dimensional fluorographs (MA Driancourt, unpublished results) follicular fluid

was not used to study between-follicle interactions. Aromatization was the end point used in most studies because this is a key follicular function increasing with growth, decreasing with atresia as well as being FSH dependent (Driancourt, 1992).

Experiment 1: Can atretic follicles exert a positive effect?

Part 1: Collection of incubation media and ovarian venous serum

The oestrous cycles of 24 Île de France ewes were synchronized by the insertion of intravaginal progestagen-impregnated sponges (Chronogest, Intervet, Angers, France) for 14 d. Following sponge removal, oestrus was detected twice daily to identify the day of oestrus (day 0). Ewes were then randomly assigned to undergo surgery either at day 6 ($n = 12$), a period when the large luteal phase follicle is likely to be healthy (Driancourt *et al*, 1991) or at day 9 ($n = 12$), a time when, in most ewes, the large luteal phase follicle is becoming atretic (Driancourt *et al*, 1991). At each time, a sample of blood (for serum) was taken from each sheep, the ewes were then laparotomized under general anesthesia induced by barbiturates and maintained by halothane in oxygen. Ovarian venous blood draining each ovary was collected (when possible), location of the corpus luteum was recorded and then the ewe was ovariectomized.

Follicles > 4 mm were dissected, measured and then incubated for 2 h in 2 ml B_2-Menezo medium as previously described by Webb *et al* (1989). At the end of culture, the medium was collected and stored frozen until assays of oestradiol and testosterone were performed (Terqui, 1978; Hochereau de Reviers et al, 1990). The intra-assay coefficients of variation were 7.7 and 11.5% for oestradiol and testosterone, respectively. Minimum detectable values were 0.02 and 0.2 ng/ml for oestradiol and testosterone, respectively. Once the results of the oestradiol assay were available, pools of all healthy (*ie* producing > 500 pg/ml/h according to Webb *et al*, 1989) or atretic (producing < 500 pg/ml/h) follicles were made and filter sterilized. Furthermore, pools of ovarian venous serum (balanced for the presence of the corpus luteum) draining the ovary with the dominant follicle (day 6) or the contralateral one, or draining the atretic follicle (some day-9 ewes) or the contralateral one were formed and filter sterilized. Protein concentrations of these samples (conditioned media and ovarian sera) were measured according to Bradford (1976) using BSA as a standard.

Part 2: Test of these biological materials on aromatization

The oestrous cycles of 8 Romanov ewes were synchronized by the technique described previously *(Part 1)*. At 30 h after sponge removal, the ewes were slaughtered and the ovaries collected. The largest follicles were dissected and measured. Following aspiration of follicular fluid, each follicle was split in 3 *(Experiment 1a)* or 4 *(Experiment 1b)* fragments of follicular walls, and put into culture (see below). In *Experiment 1a* each fragment was treated with similar protein concentrations of: (1) serum (30 µl) (fragment 1); medium conditioned by healthy follicles (300 µl) (fragment 2); or medium conditioned by atretic follicles (300 µl) (fragment 3). In *Experiment 1b,* each fragment was treated with ovarian serum draining the dominant follicle or the contralateral ovary or ovarian serum draining the atretic follicle or the contralateral ovary (30 µl) (fragments 1, 2, 3 and 4, respectively). This procedure, in which different treatments are tested on follicular walls of the same follicle, enables the use of paired tests to analyze the data.

Experiment 2: Is there a positive effect of large follicles of Booroola ewes on aromatization or cell proliferation?

Part 1: Collection of incubation media and ovarian venous blood

The oestrous cycles of 5 *F* + and 7 ++ Booroola Merinos were synchronized as described earlier. At 24 h following sponge removal, ewes were laparotomized, serum and ovarian serum together with follicles were collected and used as in *Experiment 1*. The only differences were that follicles larger than 5.0 and 3.5 mm were incubated for ++ and *F* + ewes, respectively, and that follicular health was tested once the initial incubation was completed by a 3 h culture in the presence of ^{3}H-testosterone (300 000 dpm, Du Pont de Nemours,

Les Ulis, France). Aromatization, which converts ^3H-testosterone to ^3H_2O (Gore Langton and Dorrington, 1981; Thatcher *et al*, 1991) vanishes when follicles enter atresia (Tsonis *et al*, 1984). Follicles with a number of counts in the aqueous phase similar to the assay blank were considered atretic and discarded. Several pools were then prepared for use in *Part 2*: (1) for each genotype, pools of ovarian sera or conditioned media were produced by mixing all relevant samples of all ewes; and (2) a serum pool was produced by mixing all samples of all ++ and *F* + ewes.

Part 2: Search for biological effects of these materials on aromatization *(Experiment 2a, b)* or proliferation *(Experiment 2c)*

The oestrous cycles of 30 ++ and 12 *F* + Booroola Merinos were synchronized as described earlier to generate the follicles used in *Experiment 2a* (8 ++ and 4 *F* + ewes) and *Experiment 2b* (22 ++ and 8 *F* + ewes). At 30 h following sponge removal, ewes were slaughtered and follicles > 4.5 and > 3.5 mm (++ and *F* + ewes, respectively) were used as in *Experiment 1*. *Experiment 2a* invoved 8 ++ and 10 *F* + follicles. Each follicle was cut into 3 follicular walls treated with similar protein concentrations of either a pool of ++ and *F* + serum (30 µl) (fragment 1) or 300 µl of media conditioned by healthy follicles of ++ or *F* + ewes (fragments 2 and 3, respectively). *Experiment 2b* involved 23 ++ and 19 *F* + follicles which were split into 3 fragments. These were treated with similar amounts (30 µl) of serum (pool from ++ and *F* + ewes) (fragment 1) or ovarian serum draining the ovary with a large healthy follicle of ++ or *F* + ewes (fragments 2 and 3, respectively) in the absence or presence of 10 ng/ml of oFSH. Twelve ++ and 11 *F* + follicles (from 12 ++ ewes and 5 *F* + ewes) were cultured in the absence of FSH while 11 ++ and 8 *F* + follicles (from 10 ++ ewes and 3 *F* + ewes) were cultured in the presence of FSH. The oFSH (cy 1746) used had a potency of 51 NIH FSH S_1 and its contamination by LH and TSH did not exceed 1%.

In *Experiment 2c*, the effect on granulosa-cell proliferation of coculture of small follicles with a large follicle in the presence/absence of FSH in *F* + and ++ ewes was studied. Small follicles were obtained from 8 *F* + and 10 ++ ewes, at 24 h after the end of a synchronization treatment with progestagen impregnated sponges (see above). Large follicles were obtained from 3 *F* + and 5 ++ ewes at 36 h after sponge removal. Large follicles > 5.5 mm and 4 mm (++ and *F* + ewes, respectively) and small follicles (*ie* 1–2 or 0.8 –1.5 mm in ++ and *F* + ewes) were dissected, measured and those presenting evidence of atresia when examined under a dissecting microscope were discarded.

Within each breed, small follicles were assigned to be culture either alone, in the presence of 50 ng/ml oFSH (see above), in the presence of a large follicle from the same breed, or in the presence of FSH and a large follicle. The modulatory effects of these treatments on proliferation of granulosa cells, measured by the labelling index following ^3H thymidine incorporation were measured. The rationale for selecting cell proliferation as an end point is that cell proliferation in the most typical feature of small follicles (Turnbull *et al*, 1977) while differentiated functions such as aromatase appear in larger follicles.

Follicles were incubated (5 per plate) in 24-well plates in 1 ml Menezo medium devoid of thymine and supplemented with 2 µCi/ml of tritiated thymidine (CEA, Saclay, France) for 4 h at 37°C in an atmosphere of 95% O_2. At the end of incubation, the follicles were fixed in Bouin Hollande's fixative devoid of mercuric chloride and sectioned at a 5 µm thickness. After Feulgen staining, the slides were prepared for autoradiography. After dipping in Ilford K 5 emulsion diluted 1:1 (v/v) with distilled water, they were air-dried and exposed at 4°C for 10 d, then developed and counterstained with hematoxylin. All labelled cells on the largest section of each follicle (*ie* those with more than 10 silver grains per cell) were counted. The granulosa-cell number of the section studied was obtained from antrum size, granulosa-layer thickness and granulosa-cell density. On all healthy follicles (*ie*, with < 5 pycnotic bodies in the section studied), the labelling index of the granulosa cells was calculated (number of labelled cells/total number of cells in the section).

Experiment 3: Do dominant follicles produce compounds affecting aromatization?

Follicular fluid and incubation media from healthy Ile de France follicles were collected in *Experiment 1*. *Experiment 3* involved 8 Ile de France

follicles which were split into 3 fragments, and used to compare the effects of similar amounts of proteins originating from follicular fluid or incubation medium. Serum (fragment 1), follicular fluid from large healthy follicles (fragment 2) (30 µl) and media conditioned by large follicles (fragment 3) (300 µl) were tested.

Aromatase test

All test substances (serum, follicular fluid, ovarian serum, conditioned media) were added at the beginning of culture, in 600 µl Menezo medium (experiments not involving conditioned media) or 900 µl culture medium (experiments in which one of the treatment groups used conditioned medium). The amount of protein added in the different experiments was selected to be as physiological as possible (*ie* a quarter of large follicle was incubated in the presence of around 25% of the total amount of follicular fluid present in a large follicle). Ten hours after the beginning of culture, 500 000 dpm of ^{3}H-testosterone (1β 2β^{3}H-testosterone with 75% of the label at the C1β and C2β positions and 25% of the label at the C1α and C2α positions) (Du Pont de Nemours, Les Ulis, France) were added. This 10-h lag before adding the ^{3}H-testosterone was selected because it is known to be long enough for FSH to increase (Westhof *et al,* 1989; Driancourt, 1992) or pregnant corpus luteum extract to decrease (Al Gubory *et al,* 1994) aromatization.

At the end of culture, the medium was removed and stored frozen while each follicle quarter was blotted dry and carefully weighed. The experimental design for all experiments is summarized in figure 1.

Aromatase activity was measured by the release of ^3H_2O from ^{3}H-testoserone as described by Gore Langton and Dorrington (1981) and validated by Thatcher *et al* (1991). Aromatase activity was expressed as:

$$\frac{\text{dpm } {}^3H_20}{\text{dpm } {}^3H_20 + \text{dpm } {}^3\text{H-testosterone}}$$

per mg of tissue

Assay blanks were included in all experiments. In these blanks, only 2–3% of the label was converted to $^3H_20$. These counts were deducted from the counts found in the test samples to estimate aromatase activity. Validation of this procedure demonstrated that: (1) aromatase activity was partly maintained during culture being 55% between 20 and 24 h of culture of the level at 0–4 h of culture (Driancourt, 1992); (2) aromatase activity of 2 fragments of the same follicle is highly correlated (n = 18; r = 0.65; $P < 0.01$); and (3) aromatase activity can be increased or decreased by treatment with FSH or corpus luteum extract (Driancourt, 1992; Al Gubory *et al,* 1994).

Statistical analysis

Arc sin √ transformed data (aromatase activity, labelling index) were analyzed by 1-way or 2-way ANOVA for paired measurements (aromatase activity) or for unrelated measurements (labelling indices). Size and hormonal concentrations of healthy and atretic follicles *(Experiment 1)* were compared by t tests. Results are means ± sem.

RESULTS

Experiment 1

The ewes ovariectomized at day 6 and 9 yielded 10 healthy and 2 atretic follicles (day 6) and 10 healthy and 13 atretic follicles (day 9). The mean size of the healthy follicles was significantly larger (5.52 ± 0.22 *vs* 4.70 ± 0.20 mm $P < 0.01$) than that of the atretic follicles. Healthy follicles at day 6 were marginally larger (6.02 ± 0.32 *vs* 5.04 ± 0.36 mm $P < 0.1$) than healthy day-9 follicles. Healthy follicles produced significantly more oestradiol *in vitro* than atretic follicles (10.02 ± 1.49 *vs* 0.35 ± 0.07 ng/ml/h). In contrast, testosterone production by healthy and atretic follicles was similar (healthy 2.33 ± 0.42 *vs* atretic 3.61 ± 1.0 ng/ml/h). Within healthy follicles, no day effect (day 6 *vs* day 9) was found for *in vitro* oestradiol and testosterone production. The effects on 13 follicles of similar protein concentrations of serum, and media conditioned by healthy or atretic follicles were assessed in *Experi-*

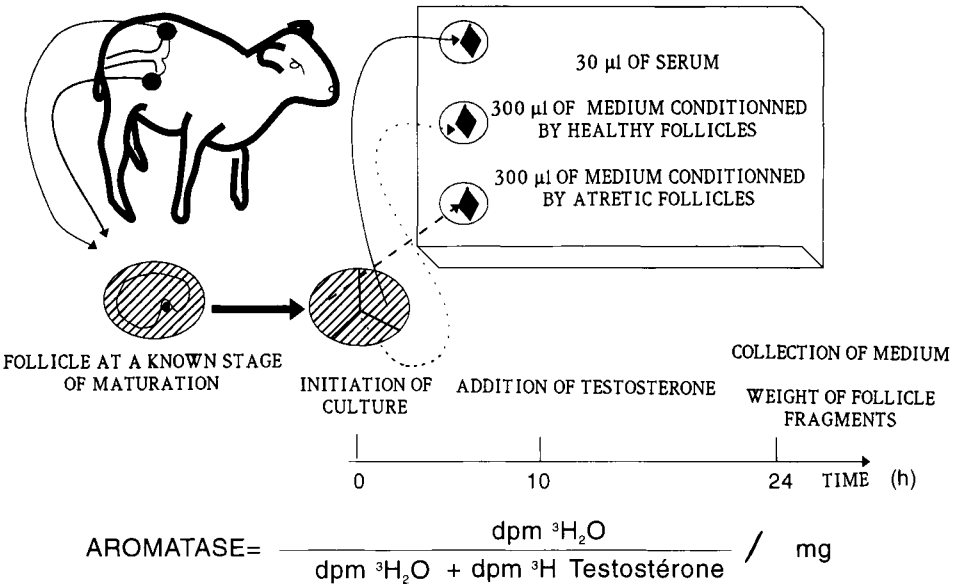

Fig 1. Experimental design used to run all aromatase tests. The design of *Experiment 1a* is illustrated.

ment 1a. No significant treatment effect could be observed and the aromatase activities of the follicular walls treated with serum, media conditioned by healthy or atretic follicles were 0.062 ± 0.009, 0.063 ± 0.009 and 0.063 ± 0.005, respectively.

These results were confirmed by *Experiment 1b,* which compared the effects of similar protein concentrations of ovarian serum draining the ovaries bearing the dominant or atretic follicles and the contralateral ones. Aromatase activity was similar in the follicular walls treated with ovarian serum from the ovary bearing the dominant follicle (0.049 ± 0.08), the contralateral one (0.052 ± 0.006), the ovary bearing the atretic follicle (0.044 ± 0.005), and the contralateral one (0.045 ± 0.006).

It can be concluded from these 2 experiments that the large atretic follicle does not secrete compound(s) with a positive effect on aromatase activity.

Experiment 2

Thirteen and 9 follicles were dissected from the 5 *F* + and 7 ++ ewes. Of these, 11 and 8 follicles were healthy as indicated by an aromatase activity over the blank values of the aromatase test. Pools of incubation media and ovarian serum originating from these follicles were used for further studies.

Experiment 2a compared the effects of similar protein concentrations of serum or media conditioned by *F* + or ++ follicles on aromatase activity. There was no significant treatment effect (serum *vs* media), no significant genotype effect (*F* + VS ++) and

no treatment x follicle type interaction (table I).

Experiment 2b tested the effects of ovarian serum (from *F* + and ++ ewes) on follicles of either genotype (*F* + and ++) in basal (no FSH) or FSH-stimulated conditions. In basal conditions, a significant genotype ($P = 0.02$) effect but no treatment effect and no genotype x treatment interaction were found (fig 2). In FSH-stimulated conditions (fig 3), similar conclusions were obtained. There was no treatment effect and no genotype x treatment interaction. A significant genotype effect was, however, detected ($P < 0.01$). It can be concluded from these 2 experiments that follicles from *F*-gene-carrier Booroola ewes do not secrete compound(s) with a positive action on aromatase activity.

Table I. Effects of similar protein concentrations of serum or media conditioned by large healthy follicles of ++ or *F* + ewes on aromatase activity of ++ or F + follicles (*n* = number of follicles studied).

	Origin of follicles	
	++ (n = 8)	*F + (n = 11)*
30 µl serum	0.035 ± 0.006	0.059 ± 0.008
300 µl medium conditioned by ++ follicles	0.048 ± 0.005	0.058 ± 0.003
300 µl medium conditioned by		

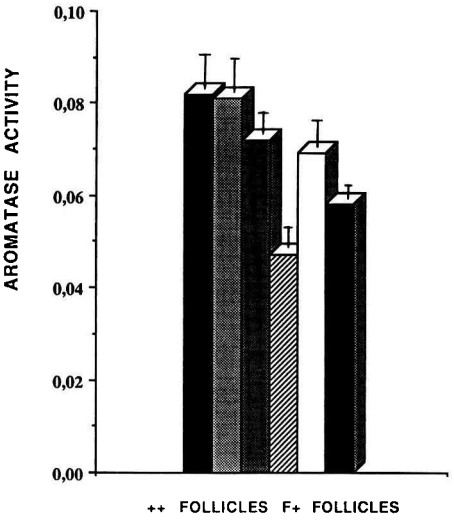

Fig 2. Effects on aromatase activity of ++ follicles (left 3 bars) or *F* + follicles (right 3 bars) of similar protein concentrations of a serum pool (*F* + and ++ ewes) or ovarian serum from ++ or *F* + ewes (30 µl of each) in the absence of FSH (*n* = 12 ++ and 11 *F* + follicles). ■ serum pool; ▨ ovarian serum ++ ewes; ▨ ovarian serum F + ewes; ▨ serumpool; □ ovarian serum ++ ewes; ▩ ovarian serum *F* + ewes.

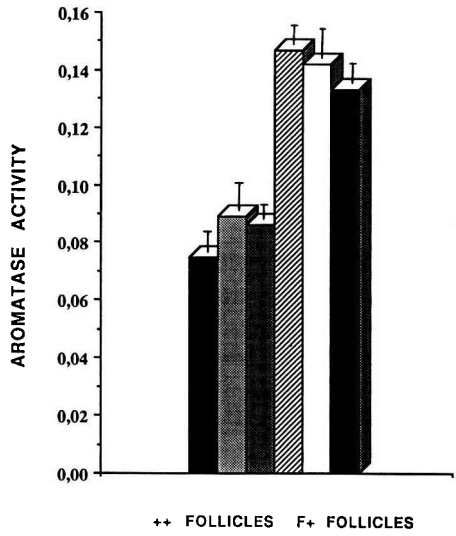

Fig 3. Effects on aromatase activity of ++ follicles (left 3 bars) or *F* + follicles (right 3 bars) of similar protein concentrations of a serum pool (same in figure 2) or ovarian serum from ++ or *F* + ewes (30 µl of each) in the presence of 10 ng/ml FSH (*n* = 11 ++ and 8 *F* + follicles). See figure 2 for legends.

Whether such follicles could modulate granulosa-cell multiplication in the absence or presence of FSH was tested in *Experiment 2c,* by coculture of small follicles in the presence or absence of a large follicle of the same breed. Of the 45 ++ and 39 *F* + small follicles dissected, 33 and 26 (++ and *F* +, respectively) were healthy. Analysis of the labelling indices by 2-way ANOVA revealed a significant breed effect ($P < 0.001$), no treatment effect and a breed x treatment interaction which just fails to achieve statistical significance ($P < 0.1$) (table II). The labelling indices of *F* + follicles were higher than those of ++ follicles ($P < 0.01$) and the increases induced by FSH treatment were larger in F + than in ++ follicles ($P < 0.05$). However, presence of a large follicle did not affect basal or FSH-stimulated granulosa-cell proliferation. It can be concluded that large follicles from both breeds do not produce compounds modulating granulosa-cell multiplication.

Experiment 3

Possible effects on aromatase activity of similar amounts of proteins from follicular fluid, serum or media conditioned by large healthy follicles were studied in *Experiment 3.* A significant treatment effect was detected ($P < 0.05$) as aromatase activity was significantly lower in the presence of follicular fluid from large follicles than in the presence of conditioned medium ($P < 0.02$) or serum ($P < 0.05$) (fig 4).

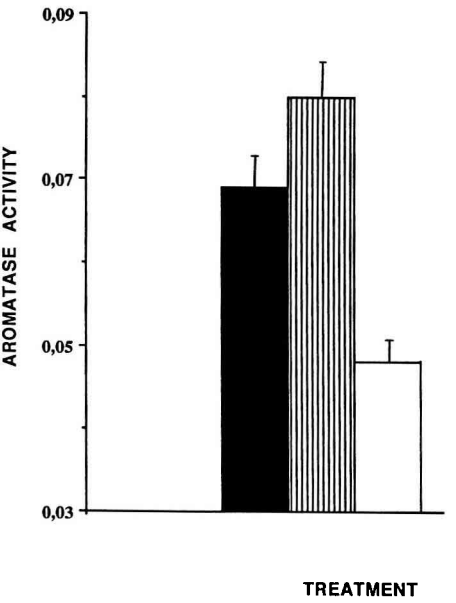

Fig 4. Effects on aromatase activity ($n = 8$ follicles split into 3 fragments) of similar amounts of proteins originating from serum, medium conditioned by large follicles, and follicular fluid from large follicles (30 µl of serum or follicular fluid, 300 µl of conditioned medium). ■ serum; ▥ conditioned medium; □ follicular fluid.

Table II. Effects of FSH and coculture with a large healthy follicle on the labelling index of the granulosa cells of small follicles of ++ or *F* + ewes (n = number of follicles studied).

	Origin of the follicles	
	++	*F +*
Alone	0.17 ± 0.14 ($n = 10$)	0.44 ± 0.15 ($n = 7$)
FSH	0.13 ± 0.03 ($n = 8$)	0.64 ± 0.30 ($n = 6$)
Large follicle	0.21 ± 0.17 ($n = 7$)	0.37 ± 0.26 ($n = 8$)
Large follicle + FSH	0.15 ± 0.15 ($n = 8$)	0.61 ± 0.25 ($n = 5$)

DISCUSSION

The main result of this *in vitro* study is that between-follicle interactions could not be demonstrated in sheep. No positive role of the large atretic follicle and no negative role of the dominant follicle on differentiation of other follicles (measured by aromatase activity) could be found. Furthermore, dominant follicles from Booroola ewes (*F*+) do not secrete compounds that stimulate cell replication or differentiation of other follicles.

The search for between-follicle regulators was conducted using ovarian serum or media conditioned by follicles. All these biological samples were collected from individual follicles at specific times of their maturation towards ovulation or atresia, in an effort to mimick the *in vivo* situation.

Throughout the cycle, large follicles develop according to a wave-like pattern with a close synchrony between atresia of the large follicle which developed during a wave and recruitment of follicles for the next wave (sheep: Smeaton and Robertson, 1971; Driancourt *et al,* 1988; Cattle: Sirois and Fortune, 1988; Savio *et al,* 1988). Two candidates for the control of the renewal of follicular waves could be: (1) FSH fluctuations which appear to occur regularly throughout the cycle in cattle (Adams *et al,* 1992) and less regularly in sheep (Miller *et al,* 1981); or (2) a stimulatory effect of the large atretic follicle when it enters atresia. Such an effect would potentiate the action of existing FSH concentrations, hence inducing an increase in aromatase activity of small follicles and starting a new wave. Such a possibility was supported by *in vivo* results in sheep (Driancourt *et al,* 1991) and cattle (Goulding *et al,* 1990). However, when the hypothesis of a positive role of the early atretic follicle was tested in sheep, neither ovarian venous blood draining the atretic follicle nor media conditioned by atretic follicles significantly altered aromatase activity. This suggests that: (1) atretic follicles do not exert local effects; or (2) they may do so, but only for a short period of time, which was not covered in this study. This second possibility is unlikely, as shown by the following observations. Firstly, *in vitro* testosterone production was similar in healthy and atretic follicles. Secondly, the size of these atretic follicles was only marginally reduced compared with healthy follicles. Such a reduction is usually observed after 1 d of atresia (Driancourt *et al,* 1987). Thirdly, *in vitro* oestradiol production, while low, was still detectable. Hence, it may be concluded that the follicles used in this study were in early atresia. The overall conclusion of these studies aiming at identifing a positive action of the atretic follicle is that it was undetectable in sheep. Follicular waves may then be induced by the existing fluctuations in FSH concentrations even if they are limited (Driancourt *et al,* 1988). Indeed, Picton and McNeilly (1991) have demonstrated that a 10% change in FSH support to follicles is able to alter the growth of large follicles significantly.

What is responsible for the high ovulation rate of the ewes carrying the Booroola gene has not yet been clarified. Higher FSH concentrations have been observed in *F* gene carriers compared with non-carriers (McNatty *et al,* 1987), while gonadotrophin-independent alterations in ovarian function have also been reported (Fry *et al,* 1988; McNatty *et al,* 1990). Compared with non-carriers, a significant difference in the way follicles grow towards ovulation in F gene carriers has been reported (Driancourt *et al,* 1985). Recruitment of follicles in the gonadotrophin-dependent phase occurs even in the presence of already dominant follicles in *F* gene carriers, while this never happens in non-carriers. Such an observation could be explained by a positive effect of the follicles growing during the early follicular phase either on cell proliferation or

aromatization. The 3 experiments performed to test this hypothesis have failed to support it. Hence, it is unlikely that an alteration in the between-follicle relationships is causing the increased ovulation rate found in the *F* gene carriers. Whether the stronger stimulatory effects of FSH on cell proliferation and aromatase activity in *F*-gene carriers play a significant role in generating higher ovulation rate requires further investigation.

One puzzling aspect of the mechanism whereby only a single follicle matures and ovulates in most breeds of sheep is the method used by this follicle to become dominant. The present study, in which media conditioned by dominant follicles did not affect differentiation of other follicles, suggests that no direct inhibitory action of this follicle occurs in sheep. This further confirms an earlier report (Driancourt *et al*, 1991). Appearance of a dominant follicle could then be produced by the fall in FSH concentrations occurring during the mid-late follicular phase. Interestingly, follicular fluid from dominant follicles contained an inhibitor of aromatase activity, which was not secreted (no activity in conditioned medium). This finding shows that the aromatase test used in the present study is sensitive enough to demonstrate treatment effects. This is in good agreement with earlier results testing effects of pregnant corpus luteum extracts (Al Gubory *et al*, 1993) or gonadotrophins (Driancourt, 1992). The demonstration of an aromatase inhibitor in follicular fluid supports earlier *in vivo* (Cahill *et al*, 1985a, b; Campbell *et al*, 1991) in sheep; it also fits the demonstration of a similar activity in primate follicular fluid and ovarian venous blood (Di Zerega *et al*, 1982). Whether the secretion of such an aromatase inhibitor in primates (compared with the lack of secretion observed in sheep) explains the tighter control of ovulation rate in the former species remains to be established.

The overall conclusion of this study is that no between-follicle interactions could be detected in sheep. In contrast an inhibitor of aromatase activity was present in follicular fluid of large follicles. Whether these conclusions also stand for other species remains to be established.

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