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Original article

Effect of short photoperiodic cycles on male genital tract and testicular parameters in male goats *(Capra hircus)*

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Summary — This study was performed in adult male goats in which seasonal variations were abolished by rapid alternations of long days and short days. These treatments have been shown previously to prevent seasonal changes in the hypothalamo–pituitary axis and to maintain testis weight and sperm production at a high level. The experimental groups were exposed for 3 years to an alternation of either a 1 month short (16 h dark; 8 h light) and 1 month long (16 L; 8 D) photoperiod (2 month cycle; $n = 5$) or of a 2 month short and 2 month long photoperiod (4 month cycle; $n = 4$). The control groups were maintained in natural photoperiodic conditions (45°N) and goats were slaughtered in the non-breeding season (end of April RS; $n = 5$) at the same period as light-treated bucks, or in the breeding season (end of September BS; $n = 6$). The total weight of the testes, the length and mean diameter of the seminiferous tubules of light-treated goats were similar to those in the breeding season, and higher than those in the non-breeding season. The total number of A_0 spermatogonia was increased by light treatments as compared to control goats in the breeding and non-breeding season. The daily production of A_1 spermatogonia, leptonene primary spermatocytes and round spermatids in light-treated goats was maintained at the peak breeding season level. The intra-testicular concentration of testosterone, total volumes of intertubular tissue and of Leydig cells, and the number of Leydig cells per testis did not differ between groups. Although the mean cross-sectional area of Leydig cells in light-treated goats was similar to this area in non-breeding season goats, it was significantly lower than that of breeding season goats. In conclusion, the rapid alternation of short and long days allowed an increase in all the germ cells from the A_0 spermatogonia onwards, which was responsible for the maintenance of high spermatogenetic activity of light-treated goats.

testis / buck / photoperiod / spermatogenesis / Leydig cells

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Résumé — Effet des traitements photopériodiques sur le tractus génital et les paramètres testiculaires chez le bouc *(Capra hircus).* *Des rythmes photopériodiques accélérés pendant 3 années consécutives abolissent les variations saisonnières de l'activité sexuelle chez les boucs. Ces traitements atténuent les changements saisonniers de l'activité de l'axe hypothalamus–hypophyse–testicule et permettent de maintenir une activité spermatogénétique élevée. Pour étudier l'effet de ces traitements photopériodiques sur les paramètres testiculaires, 4 groupes de boucs de race Alpine et Saanen sont utilisés. Les groupes expérimentaux sont soumis à une alternance de 1 mois de jours longs (16 h de lumière ; 8 h d'obscurité) et de 1 mois de jours courts (8 L : 16O ; lot : 2-mo ;* n = *5) ou 2 mois de jours longs et 2 mois de jours courts (lot : 4-mo ;* n = *4). Les groupes témoins ont été placés sous les variations naturelles de la photopériode (45° latitude nord). Les 2 lots expérimentaux et un groupe témoin (lot : repos sexuel RS ;* n = *5) sont abattus fin avril. L'autre lot témoin est abattu fin septembre (lot : activité sexuelle BS ;* n = *6). Le poids testiculaire, la longueur et le diamètre des tubes séminifères sont similaires pour les animaux des 2 lots expérimentaux et du lot BS, mais supérieurs à ceux des boucs du lot RS. Le nombre total de spermatogonies A_0 est augmenté dans les lots traités par rapport aux 2 lots témoins. La production quotidienne de spermatogonies A_1, de spermatocytes primaires leptotènes et de spermatides rondes dans les lots traités est semblable à celle observée dans le groupe BS. La concentration intratesticulaire de testostérone, le volume total du tissu intertubulaire et celui des cellules de Leydig, et le nombre de cellules de Leydig par testicule ne sont pas différents entre lots. La surface de section des cellules de Leydig est identique entre les 2 lots traités et le lot RS mais inférieure dans le lot BS. Il est conclu que l'alternance rapide entre les jours longs et les jours courts augmente le nombre de cellules de la lignée germinale depuis les spermatogonies A_0 souches de réserve et permet le maintien d'une activité spermatogénétique élevée chez les boucs traités.*

testicule / bouc / photopériode / spermatogenèse / cellules de Leydig

INTRODUCTION

Alpine and Saanen male goats naturally exhibit large seasonal variations in LH and testosterone secretion, testis weight, quantitative and qualitative sperm production (Delgadillo and Chemineau, 1992; Delgadillo *et al,* 1991, 1992). Maximum plasma levels of both hormones and sperm production occur in autumn and winter (Saumande and Rouger, 1972; Delgadillo *et al,* 1991; Delgadillo and Chemineau, 1992), and these seasonal variations are mainly entrained by photoperiodic changes (Branca and Cappai, 1989; Delgadillo *et al,* 1991; Delgadillo and Chemineau, 1992).

In male sheep and goats subjected to rapid photoperiodic changes, the seasonal fluctuations in LH and testosterone release were abolished and maximum plasma LH and testosterone concentrations in light-treated males never reached those observed in control animals during the breeding season (Almeida and Pelletier, 1988; Delgadillo and Chemineau, 1992). These rapid photoperiodic alternations prevented the negative feedback effect of high testosterone secretion and therefore maintained high testis weight and sperm production (Almeida and Pelletier, 1988; Delgadillo and Chemineau, 1992). Indeed, light treatments allowed experimental groups to produce more spermatozoa per ejaculate, even during the natural breeding season of the control bucks. The total number of spermatozoa per ejaculate in a second experimental year was higher in the 2 and 4 month groups than in the controls (Delgadillo *et al,* 1991). This difference was also recorded at the end of the third experimental year during the daily sperm output test (Delgadillo *et al,* 1993).

The aim of this study was to determine the effects of 2 month and 4 month light cycles on genital tract, seminiferous tubule and Leydig cell parameters and on intra-

testicular testosterone concentration and compare them with testes of bucks examined during and out of the breeding season.

MATERIALS AND METHODS

Animals

Nine adult Alpine and Saanen male goats were submitted over 3 consecutive years to rapid photoperiodic changes. They were the same males as described in Delgadillo *et al* (1992, 1993). The first group, 2 months (2-mo, $n = 5$ animals), was subjected to 1 month of long days (16 h of light/8 h of darkness) and to 1 month of short days (8 h of light/16 h of darkness). The second group, 4 months (4-mo, $n = 4$), was subjected to alternations of 2 months of long and 2 months of short days. They were compared with 2 groups of male goats subjected to natural photoperiodic changes, taken either at the end of April ($n = 5$) during the non-breeding resting season (RS) or during the breeding season (BS) at the end of September ($n = 6$).

Goats of 2-mo, 4-mo and RS groups were slaughtered at the end of the third experimental year (end of April, at the end of a short-day period) and BS goats were slaughtered in October. Immediately after slaughter, testes, epididymis and seminal vesicles were weighed. A small sample (1–2 cm^3) of one testis per buck was fixed in Bouin–Hollande solution and processed for histological observation. The second testis was kept frozen at –20°C until assayed for intra-testicular testosterone.

Histology

Histological analysis was performed on 10 μm thick sections as previously described (Hochereau-de-Reviers *et al,* 1992). Seminiferous tubule (volume, total length, diameter germinal and Sertoli cell number) and intertubular tissue (total volume, Leydig cell number and volume) parameters were determined. The relative volumes of intertubular tissue and seminiferous tubules in testicular parenchyma, and of Leydig cells and blood and lymph vessels in intertubular tissue were determined with a 25-point ocular integrator on 20 fields per animal at magnifications x 200 and 800, respectively. The mean cross-sectional area of seminiferous tubules, Sertoli cell nuclei and Leydig cells was measured with a planimeter program (Apple software) respectively at magnifications x 40 and 1 000 respectively. The nuclei of Sertoli, A_0 and A_1 spermatogonia (stages 6 to 1 according to Ortavant, 1959), leptotene primary spermatocytes (stages 1 and 2) and round spermatids (stages 6–1) were counted on 10 cross-sections of seminiferous tubules per animal. The number of Sertoli cells and germ cells per section was corrected for the nuclear size and section thickness as described by Abercrombie (1946), assuming that nuclei were spherical in nature.The total length of the seminiferous tubules per testis was then calculated according to the equation of Attal and Courot (1963). The total numbers of Sertoli, germinal and Leydig cells were calculated as described in Hochereau-de-Reviers *et al* (1992), and the daily production of germ cells was then calculated from their total numbers per testis divided by the mean duration of the seminiferous epithelium cycle, *ie* 9.8 d in the male goat (Derashri *et al,* 1992).

Intra-testicular testosterone content

To measure testosterone, a sample *(ca* 4.3 g) of testicular parenchyma was homogenized in 12 ml distilled water. The homogenates were incubated for 30 min at ambient temperature with 2 000 cpm [^{3}H] testosterone to estimate the yield of testosterone. Steroids were extracted in 5 vol cyclohexane/ethyl acetate (1:1, vol/vol) and testosterone levels were measured by radioimmunoassay as previously described (Delgadillo and Chemineau, 1992).

Statistics

Testicular and epididymal weights were compared by Student's *t*-test. Histological parameters and intra-testicular testosterone content were compared either by analysis of variance or by Mann–Whitney non-parametric *U*-test (Statview and Superanova softwares, Abacus concept, CA, USA).

RESULTS

Genital tract and intra-testicular testosterone content

Testis weights did not differ between 2-mo, 4-mo and BS goats and equalled, on average, 174 g (table I). However, they were significantly heavier (117 g) than those from RS goats, +62% ($P < 0.01$), +55% ($P < 0.05$) and +28% ($P < 0.05$) for 2-mo, 4-mo and BS, respectively. The mean epididymal weight of light-treated goats (2-mo and 4-mo groups combined) was significantly heavier than those maintained in natural light conditions (BS and RS groups combined) (+24%; $P < 0.05$). Seminal vesicles weight, mean intra-testicular testosterone concentrations and mean total testosterone content did not differ significantly between groups, but the variation coefficient was higher in RS and BS bucks than in light-treated animals.

Intertubular tissue parameters

The total volume of intertubular tissue, of blood vessels and lymph, of Leydig cells and the total number of Leydig cells per testis did not differ significantly between groups (table II). The cross-sectional area of Leydig cells was higher in BS animals that in RS, 2-mo and 4-mo groups (+39, +20 and 30%, respectively) ($P < 0.05$).

Table I. Testicular, epididymal, seminal vesicular weight and intra-testicular concentration of testosterone of Alpine and Saanen male goats subjected to 2 month (2-mo) and 4 month (4-mo) light cycles or to natural photoperiodic changes and slaughtered during breeding (BS) or resting (RS) seasons (m ± sd).

Parameter	*2-mo*	*4-mo*	*BS*	*RS*
Number of goats	5	4	6	5
Mean testicular weight (g)	190 ± 44[a]	181 ± 44[a]	150 ± 52[a]	117 ± 5[b]
Mean epididymal weight (g)	38.1 ± 6.3[a]	33.1 ± 7.5[a]	28.7 ± 9.6[b]	29.2 ± 2.6[b]
Total seminal vesicular weight (g)	16.3 ± 3.6[a]	10.8 ± 2.2[a]	18.5 ± 5.3[a]	14.9 ± 4.4[a]
Intra-testicular concentration of testosterone (ng/g tissue)	44.9 ± 19.0[a]	53.9 ± 14.2[a]	104.1 ± 95.2[a]	78.1 ± 67.3[a]
Coefficient of variation (%)	42	26	91.5	73.6
Total intra-testicular content of testosterone (µg/testis)	8 531 ± 836	9 756 ± 625	15 615 ± 4 950	9 138 ± 337

For each parameter, numbers with different superscripts differed significantly ($a \neq b$: $P < 0.05$).

Table II. Intertubular tissue and Leydig cells parameters of Alpine and Saanen male goats subjected to 2 month (2-mo) and 4 month (4-mo) light cycles or to natural photoperiodic changes and slaughtered during breeding (BS) or resting (RS) seasons (m ± sd).

Parameter	*2-mo*	*4-mo*	*BS*	*RS*
Number of goats	5	4	6	5
Volume/testis				
Intertubular tissue (cm^3)	31.6 ± 9.1^a	34.5 ± 9.4^a	25.0 ± 12.7^a	28.1 ± 3.1^a
Blood and lymph vessels total volume (cm^3)	7.8 ± 2.4^a	6.9 ± 2.4^a	5.7 ± 3.1^a	7.0 ± 1.9^a
Leydig cells				
Total volume/testis (cm^3)	0.9 ± 0.3^a	0.8 ± 0.2^a	0.8 ± 0.4^a	0.9 ± 0.3^a
Numbers/testis (x 10^8)	6.6 ± 2.4^a	7.7 ± 2.1^a	4.9 ± 2.4^a	8.2 ± 2.0^a
Cross-sectional area (μm^2)	43.7 ± 8.1^a	40.3 ± 6.8^a	52.3 ± 9.5^b	37.7 ± 3.0^a

For each parameter, numbers with different superscripts differed significantly ($a \neq b$: $P < 0.05$).

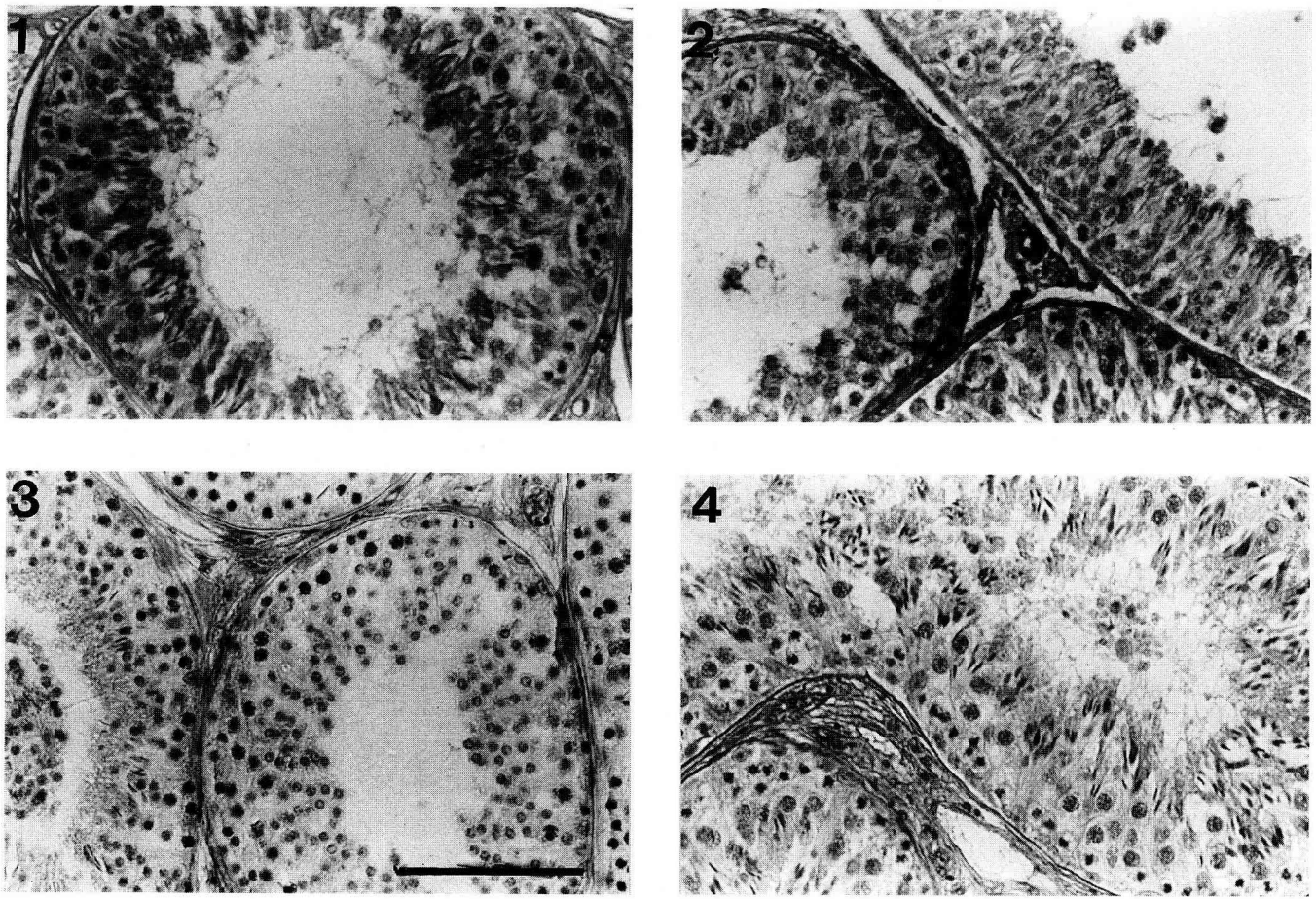
Seminiferous tubules parameters and germ cell parameters (table III, figs 1–4)

The length of seminiferous tubules per testis was not different between 2-mo, 4-mo and BS groups but it was significantly higher than in RS group (+36, +38 and +34%, respectively) ($P < 0.01$) (table III, figs 1–4). The mean diameter of the seminiferous tubules was not different between 2-mo and 4-mo groups as well as between BS and RS groups, but the mean diameter of seminiferous tubules of the 2-mo group was greater than that observed in BS and RS groups (+13 and +18%, respectively) ($P < 0.01$). The total number of Sertoli cells per testis (corrected for nuclear size) was similar in animals of all groups (mean = 16 x 10^8). The mean cross-sectional nuclear area of Sertoli cells did not differ significantly between treatments (mean = 74.7 μm^2).

The total number of A_0 spermatogonia per testis was similar in 2-mo and 4-mo animals but was significantly ($P < 0.05$) higher than that of RS and BS animals (+82 and +59%, respectively). The daily productions of A_1 spermatogonia, leptotene primary spermatocytes and round spermatids did not differ between 2-mo, 4-mo and BS groups. They were significantly higher than the values observed in RS groups (A1: $P < 0.05$; primary spermatocytes: $P < 0.001$; and round spermatids: $P < 0.01$). By combining the photoperiod-controlled and BS groups, the daily productions of A_1 spermatogonia was 85%, leptotene primary spermatocytes 125% and round spermatids 67% higher than the corresponding productions in RS goats.

DISCUSSION

The present results showed that in male goats, short light cycles enhanced the total number of germ cells from A_0 spermatogonia onwards and maintained a high spermatogenetic activity at the end of the 3 experimental years. Such high spermatogenic efficiency observed after 3 years of



Figs 1–4. Goat seminiferous epithelium (same magnification: bar = 100 μm) 1: sexual season goat; 2: non-breeding season goat; 3: 2-month light-treated goat; 4: 4-month light-treated goat. 1 and 4: stage 3 of the seminiferous epithelium cycle, just after elongation of spermatids. 2 and 3: beginning of stage 1, just after spermiation. Note the paucity of germ cells, mainly round spermatids, in seminiferous tubules of the non-breeding season goat.

Table III. Seminiferous tubules and germ cells parameters of Alpine and Saanen male goats subjected to 2 month (2-mo) and 4 month (4-mo) light cycles or to natural photoperiodic changes and slaughtered during breeding (BS) or resting (RS) seasons (m ± sd).

Parameter	*2-mo*	*4-mo*	*BS*	*RS*
Number of goats	5	4	6	5
Seminiferous tubules				
Total volume (ml)	162 ± 37	143 ± 37	127 ± 40	87 ± 7
Length/testis (m)	2 175 ± 372^{a}	2 206 ± 591^{a}	2 136 ± 664^{ab}	1 596 ± 127^{b}
Mean diameter (μm)	250 ± 19^{a}	230 ± 19	221 ± 15^{c}	211 ± 11^{c}
Lumen mean diameter (μm)	47.8 ± 7.6	48.3 ± 5.6	45.0 ± 5.7	41.8 ± 4.4
Sertoli cells				
Total No/testis (x 10^8)	17.1 ± 4.2^{a}	18.2 ± 4.9^{a}	15.4 ± 6.3^{a}	14.0 ± 2.1^{a}
Nuclear area (μm^2)	70.9 ± 9.7^{a}	79.1 ± 7.6^{a}	79.0 ± 13.0^{a}	69.8 ± 5.6^{a}
Germ cells				
Total number A0 spermatogonia/testis (x 10^8)	2.6 ± 1.2^{a}	2.5 ± 1.5^{a}	1.4 ± 0.7^{b}	1.6 ± 0.5^{b}
Daily production/testis				
A1 spermatogonia (x 10^7)	3.1 ± 1.5^{a}	2.9 ± 1.5^{a}	2.9 ± 1.3^{a}	1.6 ± 0.2^{b}
Leptotene primary spermatocytes (x 10^9)	0.9 ± 0.2^{a}	0.9 ± 0.2^{a}	0.9 ± 0.3^{a}	0.4 ± 0.1^{c}
Round spermatids (x 10^9)	2.3 ± 0.3^{a}	2.0 ± 0.6^{a}	2.2 ± 0.6^{a}	1.3 ± 0.2^{c}

For each parameter, numbers with different superscripts differed significantly ($a \neq b$: $P < 0.05$; $a \neq c$: $P < 0.01$).

experiment may explain the very high sperm production reported previously in the same bucks treated with light as compared to control bucks (Delgadillo *et al,* 1992). Indeed, on the ninth consecutive day of semen collection, the mean daily sperm output (DSO) in both light-treated groups (2-mo: 3.68 ± 0.59 x 109; 4-mo 6.25 ± 0.61 x 10^9 spermatozoa/d) was significantly higher than the RS group (2.96 ± 0.36 x 10^9 spermatozoa/d; $P < 0.05$) (Delgadillo *et al,* 1993). A similar phenomenon was also observed in Ile-de-France rams subjected to short photoperiodic cycles (Pelletier and Almeida, 1987; Chemineau *et al,* 1988). The major effect of the light treatments was detected in the germinal cells of the seminiferous tubules with quantitative variations of germ cells, the total number of Sertoli cells remaining unaffected. In the light-treated bucks used here, some endocrine parameters are known since these males were blood sampled in various occasions before slaughter (Delgadillo and Chemineau, 1992). One of the main feature of such light-treated males was the consistent modification in the frequency of pulsatile release of LH, because this gonadotrophin activity was driven by rapid photoperiodic changes and not by annual changes as in control males. The

frequency of LH release was higher most of the year, especially during the rest season when LH pulsatility was low in control males, but in reverse, during the 1 or 2 months of the full breeding season (around September), control males had a higher pulsatility than light-treated males. In the 2-mo group, mean LH concentrations were similar in long and short days, while, in the 4-mo group, these concentrations were lower in long than in short days. However, these values never reached the LH levels observed in the BS group (Delgadillo and Chemineau, 1992). Testosterone secretion may also play an important role in such phenomenon. In spite of the absence of differences between groups in the intra-testicular content of testosterone, which was probably due to the very high variability in testosterone concentration and content of BS and RS groups, a tendency towards a lower testosterone concentration in the testes of light-treated bucks as compared to that of control bucks could be detected. In fact, the mean values of plasmatic testosterone concentrations obtained in both treated groups in weekly blood samples over 2 consecutive years (2-mo: 10.6 ± 1.8 ng/ml; 4-mo: 12.0 ± 1.8 ng/ml) were significantly lower than those of the BS group (19.4 ± 1.5 ng/ml) (Delgadillo and Chemineau, 1992). This relatively low LH secretion in light-treated animals, by moderately stimulating the Leydig cells, induced the maintenance of an intermediate cell volume in between breeding season and rest season. This intermediate volume of Leydig cells and the moderate frequency of LH release determined the moderate testosterone release. This possibly reduced the steroid feedback effect on LH secretion (Pelletier and Almeida, 1987; Almeida and Pelletier, 1988). As a consequence, in light treated-groups, the moderate LH and testosterone release maintained testicular weight and spermatogenic activity from spermatogonial division to spermiogenesis at the level observed during the natural breeding season.

The cellular characteristics of Leydig cells observed here correspond to those observed in rams subjected to alternating increasing or decreasing 2 month light cycle regimen (Hochereau-de-Reviers *et al,* 1992) or that observed after 3 month treatments of constant short days (Hochereau-de-Riviers *et al,* 1985). The resulting moderate and less variable total intra-testicular testosterone content maintained a nearly constant level of stimulation of the seminiferous epithelium (and of the Sertoli cells). This could explain the absence of seasonal depletion of spermatogenesis which normally occurred in the non-breeding season and affected spermatogonial divisions, meiotic processes and spermiogenesis, inducing depletion of sperm production. This relatively constant intra-testicular level of testosterone corresponds to the situation observed during prepubertal testis growth when the stem spermatogonia developed (Monet-Kuntz *et al,* 1984). In the light-treated bucks, the first step to be stimulated is the stem cell compartment where both A0 and A1 spermatogonia populations were increased, as observed previously in the light-treated rams (Hochereau-de-Reviers *et al,* 1985, 1992). Normally during the sexual season, A0 spermatogonial population decreased when A1 spermatogonial one increased (Hochereau-de-Reviers *et al,* 1976, 1992), probably by differentiation of the A0 into A1 spermatogonia. In addition to a moderate intra-testicular level of testosterone, different factors could be suspected to be modified in this model. FSH was not assayed because of absence of accurate FSH radioimmunoassay in the goat, but we can suspect that FSH secretion was increased by the treatment. In ram, the passive immunisation against β-FSH decreased the B2 spermatogonia and the subsequent production of leptotene spermatocytes (Courot *et al,* 1985). In mice, FSH stimulates the production by Sertoli cells of steel factor (SLF or SCF) which is the ligand of c-kit; this factor stimulates the proliferation of

A spermatogonia in a dose-dependent manner (Rossi *et al,* 1993). The degradation of c-kit is enhanced by TGFβ1 (de Vos *et al,* 1993), the secretion of which is negatively regulated in Sertoli cells by FSH (Benhamed *et al,* 1988). So FSH could control the proliferation of type A spermatogonia by at least 2 mechanisms: increase in SCF secretion and decrease of its ligand degradation. A second set of factors, which could play a role in this mechanism, includes the activins/inhibin (Mather *et al,* 1992, 1994). During the seasonal cycle, changes in the plasma concentrations of inhibin occurred in parallel with variation in testis weight. The weekly changes in the plasma concentrations of inhibin are positively correlated with the changes in the plasma FSH concentrations during activation or regression of the testis. During full breeding season (maximum testis weight), inhibin exerts a negative feedback on FSH secretion, then a negative correlation is observed between FSH and inhibin (Lincoln and MacNeilly, 1989). In the rat, FSH regulates mRNA of the α- but not β-B subunit of inhibin (Krummen *et al,* 1989). Activin controls the proliferation of rat spermatogonia (Mather and Krummen, 1992; Kaipia *et al,* 1992, 1994). Furthermore, an activin β-neutralizing antiserum blocks activin β-stimulated ^{3}H-thymidine incorporation in spermatogonia (Mather *et al,* 1994). Activin receptors (Act RII) were detected in type A spermatogonia in rodents (Kaipia *et al,* 1992, 1994).

In conclusion, the rapid alternations of short and long days prevent the regression of the Leydig cells, promote the proliferation of germ cells from A0 reserve stem spermatogonia onwards and maintain a high production of spermatids.

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