Sciences & Technologie **C – N°33** Juin (2011), pp.9-15.

PATTERNS OF CIRCULATING FSH AND LH IN OULED DJELLEL EWE LAMBS DURING POSTNATAL DEVELOPMENT

Reçu le 04/03/2009 – Accepté le 13/12/2010

Résumé

Le plasma a été collecté de 150 agnelles d'Ouled Djellel nées en septembre à l'âge de 0 (< 24 heures), 1, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26 semaines, et à 1 an. Quatre agnelles ont été abattues à chacun des âges précédents, les ovaires pesés et les follicules vésiculaires. Trois mm ont été comptés et leurs diamètre mesurées sur les sections histologiques de l'ovaire. La concentration des deux hormones FSH et LH a augmenté de la naissance à l'âge de 10 semaines, puis elle a diminué pour atteindre des valeurs semblables à celle mesuré chez les agnelles (sexuellement mûres) âgé d'un an. Mais, l'hormone LH a augmenté une autre fois à l'âge de 24 semaines pour marquer le pic préovulatoire. Les variations de la concentration de ces hormones sont accompagnées d'une première augmentation du poids des ovaires et du diamètre maximal des follicules à l'âge de10 semaines, et d'une deuxième augmentation des caractères ovariens à l'âge de 18 semaines. Les résultats indiquent que la brebis d'Oulled Djellel a atteint précocement la puberté à l'age de 24 semaines et qu'une relation existe entre les variations plasmiques de la concentration FSH et LH et les changements dans la population des follicules vésiculaires en fonction de l'âge.

***Mots clés**: Ouled djellel, FSH, LH, Follicules Vésiculaires, Développement postnatal.*

Abstract

Plasma was collected for hormone measurement from 150 Ouled Djellel ewe lambs, born in September, at 0 (< 24 hours), 1, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, and 26 weeks, and at 1 year of age. Four of the ewe lambs were slaughtered at each of the time points. The ovaries of the slaughtered lambs were weighed; antral follicles≥3mm in diameter were counted and their size was measured on histological ovarian sections. Concentrations of follicle stimulating hormone (FSH) and luteinizing hormone (LH) in plasma increased from birth to 10 weeks of age, and then declined to levels similar to those of yearling, sexually mature ewes. The level of LH then rose again to reach a second peak at 24 weeks of age, which represented the preovulatory LH surge. The variation in the concentrations of the hormones was accompanied by an initial increase in ovarian weight and maximum follicle diameter at 10 weeks of age, and a second increase in these ovarian characteristics at 18 weeks of age. These data suggest that Ouled Djellel ewes reach puberty early, at 24 weeks of age, and that there is a relationship between the variations in plasma concentrations of FSH and LH and the changes in follicular populations of antral follicles at certain ages.

***Keywords**: Ouled djellel, FSH, LH, Antral follicles, postnatal development*

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ملخص

أخذ ت البلازمة من 150 نعجة أولاد جلال مولودة في سبتمبر عند عمر 0 (أقل من 24 ساعة)، 1، 4، 6، 8، 10، 12، 14، 16، 18، 20، 22 ،24 ، 26 أسبوع و سنة من العمر . ذ بحت 4 نعجات عند كل عمر من الأعمار السابقة و وزنت المبايض وعد ت الحويصلات ذات التجويف ≤3مم و قيست أقطارها فوق المقاطع النسيجية للمبيض. ارتفع تركيز الهرمونين FSH وLH من فترة الولادة إلى 10 أسابيع من العمر ثم انخفض إلى نفس المستوى المقاس عند النعجة ذات السنة من العمر و البالغة جنسيا و ارتفع مرة أخرى إلى قمة ثانية عند 24 أسبوع من العمر. نمثل قمة LH عند 24 أسبوع قمة ما قبل التبويض. كانت هده التغيرات في تركيز هدين الهرمونين مصحوبة بزيادة أولى في وزن المبيض و القطر الأعظمي للحويصلة عند 10 أسابيع من العمر و زيادة ثانية في هده الخصائص المبيضية عند 18 أسبوع من العمر. تد ل النتائج على أن النعجة أولاد جلال قد نضجت جنسيا مبكرا عند 24 أسبوع من العمر و توجد علاقة بين التغيرات في التركيزات البلازمية FSH وLH و التغيرات في مجموعة الحويصلات ذات التجويف مع العمر.

***الكلمات المفتاحية** : أولاد جلال، FSH، LH، الحويصلات ذات التجويف، التطور ما بعد الولادة*

Follicle stimulating hormone (FSH) and luteinizing hormone (LH) are required for follicular growth, maturation and steroidogenesis in ovarian mammals. The functions of these hormones have been reviewed in mammalian species [1, 2, 3, 4], and particularly in sheep and cattle [5, 6, 7]. Postnatal development of the ovaries and the variations in FSH and LH levels during development are of great importance in determining the reproductive capacity of all mammals. It has been proposed that determination of plasma concentrations of FSH and LH during the postnatal period enables evaluation of the physiological age of puberty, which is characterized in ewes by the first LH surge [8-12].

Many studies have shown that different breeds of sheep attain puberty at different ages. For example Debouillet, Dorset, Suffolk, D'man, and Timahdite ewe lambs reach puberty at 199 [13], 153 [14], 224 [10], 219 [15], and 445 [15] days, respectively. Variations in the patterns of plasma FSH and LH from birth to sexual maturity have not been studied to date in Ouled Djellel sheep, and the timing of the onset of puberty has not been established in this breed.

The objectives of the present study were: 1) to characterize patterns of circulating gonadotropins, FSH and LH, from birth to sexual maturity, 2) to determine the age at which the first ovulation and first LH surge occur, in order to estimate the physiological age of puberty and sexual maturity, and 3) to investigate the changes in follicular populations of antral follicles ≥3mm in diameter and establish their relationship with the variations in plasma concentrations of FSH and LH.

MATERIAL AND METHODS

Animals and experimental procedures

Studies were carried out on 150 ewe lambs born in late September from 3- to 4-year old crossbred Ouled Djellel sheep reared at the experimental station of Oum El Bouaghi. The oestrous cycles of the mothers had been synchronized by a 14-day treatment with intravaginal sponges containing 40mg of flurogestone acetate (Syncropart sponges). This synchronization gave us the opportunity to obtain blood samples from the required number of ewe lambs with approximately the same date of birth. After weaning at 16 weeks of age, the ewe lambs remained under natural conditions throughout the study.

The ewe lambs were fed on a standard ration**.** Four ewe lambs were killed at 0 (<24 hours), 1, 4, 6, 8, 10, 16, 18, 20, 22, 24, and 26 weeks after birth, and four at 1 year. The paired ovaries were weighed, fixed in Bouin-Holland's solution, embedded in paraffin wax, and serially sectioned at a thickness of 10µm. One section on five was mounted and the slides were stained with haematoxylin and eosin and examined using a projection microscope. The number of antral follicles ≥3mm was counted, using the nucleus of the ovum as a marker. The diameter of all antral follicles was measured microscopically, and the mean of two measurements was recorded. In addition, the diameter of the largest follicle was measured macroscopically. In the absence of follicles ≥3mm in diameter, the maximal follicular diameter was calculated. The boundary of the follicle was defined by the membrana granulosa.

Plasma concentrations of FSH and LH were determined in blood samples taken from 10 ewe lambs at 0 (< 24 hours), 1, 4, 6, 8, 10, 16, 18, 20, 22, 24, and 26 weeks, and 1 year, after birth. The ten ewe lambs sampled on each occasion included the four animals that were killed at that age. Each lamb was sampled only once. Blood samples were collected from the jugular vein into 10ml heparinized vacutainers between 9 and 10 am**.** After centrifugation (800g, 15minutes), plasma was stored at –20°C until analysis.

Hormone assays

Plasma concentrations of FSH and LH were determined at the hormonal assay laboratory of Research Unit UMR6175 (Physiology of Reproduction and Behaviour, INRA Nouzilly, France).

The concentration of FSH was determined using a radioimmunoassay (RIA) kit supplied by the National Hormone and Pituitary Programme (NHPP) of the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK). The ovine FSH (oFSH) used for iodination was oFSH NIDDK-oFSH-I-SIAFP-2 AFP. The oFSH reference preparation wasNIH-oFSH-RP2 (generously supplied by Dr. Parlow), and the oFSH antiserum was anti oFSH NIDDK-anti-oFSH (used at a final dilution of 1/80 000). The volume of plasma used was 100µl, and each sample was assayed in duplicate. The sensivity of the assay was 0.1ng/ml. The range of the standard curve was 0.1 to 6.4ng/ml. The intra-and inter-assay coefficients of variation (CV) were 10.6 and 9.5%, respectively.

The concentration of LH was measured using a sandwich enzyme linked immunosorbent assay (ELISA) developed and validated in UMR6175. Ovine LH was provided by Dr. Yves Comparnous (UMR6175, Nouzilly, France). The first monoclonal antibody (anti-β oLH518B7) was obtained from Dr. Jan Roser, Department of Animal Science, University of California, Davis, CA, USA [16] and was used at a dilution of 1/3200. The second antibody (anti-α antibody, MCA 1026) was purchased from Serotec [17].

The sensitivity of the assay was 0.1ng/ml. The range of the standard curve was 0.1 to 6.4ng/ml. The volume of plasma that was assayed was 20µl, and samples were assayed in simple**.** The intra-and inter assay coefficients of variation (CV) were 11.3 and 7.9%, respectively.

Statistical analysis

All data were statistically analysed using the Student's t-test and reported as mean ± standard error of the mean (SEM).

RESULTS

Levels and patterns of circulating FSH and LH during the postnatal period in ewe

The mean plasma levels of FSH and LH during the postnatal period are shown in figure1. During the first week after birth, mean levels of both gonadotropin hormones in lambs were lower than those at other ages (P<0.003 for FSH, P<0.05 for LH). Subsequently, an increase occurred in both FSH and LH from 4 to 8 weeks after birth (P<0.03 for FSH, P<0.05 for LH). The highest average hormone levels during the period studied were obtained in 10-week-old animals for FSH (P<0.05) and in 24-week-old animals for LH (P<0.02). However, in 10-week-old animals, LH plasma concentrations attained levels comparable to those found in 18- and 26-week-old animals (P>0.05).

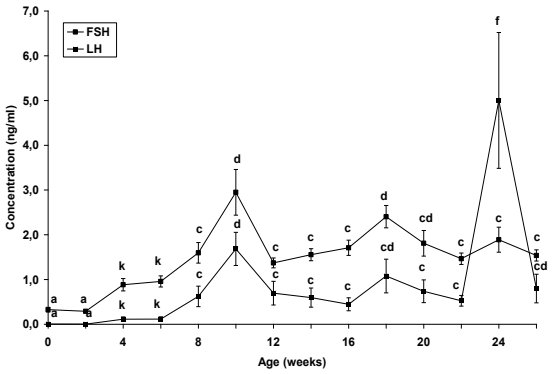

Figure 1: Peripheral plasma concentrations of FSH and LH sampled at different ages in Ouled Djellel ewe lambs (mean ± SEM, n=10). Ten ewe lambs; including the four killed animals, were sampled at each age. Average values with different letters differ significantly (P<0.05).

Plasma levels of both FSH and LH showed no differences at 8, 12, 16, 20, 22, and 26 weeks when compared with the levels in yearling ewe lambs (48 weeks old; 1.503 ± 0.222 for FSH, 0.615 ± 0.148 for LH). We noted that, at 24 weeks of age, the average concentration of LH increased by a factor of 9.54 over that at the previous age. This important increase represents the preovulatory LH surge. This suggestion was supported by the presence of a corpus luteum in histological ovarian sections obtained from one and two ewe lambs at 24 and 26 weeks after birth, respectively. In addition, ovaries at these ages were characterized by rapid maturation. The peak ovulatory LH was detected in only one ewe (18ng/ml).

Paired ovarian weights in ewe lambs during the postnatal period

The average ovarian weights of Ouled Djellel ewe lambs are presented in figure 2. The average weight of paired ovaries was lowest at birth and in 1-week-old animals. Following this, there was a significant increase at 4 and 6 weeks after birth (P<0.0002). The ovarian weight in 8- and 10-week-old animals was 11.4 and 11.9–fold higher, respectively, than the weight at birth. At 12 and 14 weeks, the weight was significantly (P<0.00002) less than at 8 and10 weeks. The ovarian weight continued to increase after these ages, and the highest weight (2.13 ± 0.07g) was recorded at 1 year of age (P<0.0002).

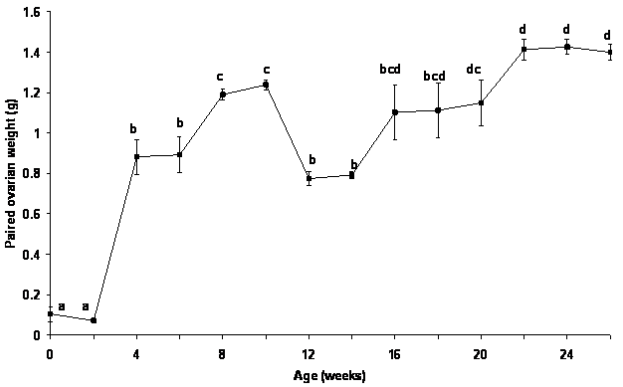

Figure 2: Average weight of paired ovaries recorded in Ouled Djellel ewe lambs at different ages (± SEM, n=4). Four animals were killed at each age. Average values with different letters differ significantly (P<0.05).

Total follicle numbers and maximum follicle diameters of antral follicles ≥3mm in diameter during the postnatal period in ewe lambs

The numbers of follicles ≥3mm in diameter did not change (P>0.05) from birth to 10 weeks of age, then increased between the 10th and 12th weeks, decreased between the 14th and 16th weeks, and subsequently increased between the 16th and 18th weeks (P<0.05, figure 3). There were no significant changes in the numbers of follicles ≥3mm in diameter from the 22nd to the 26th weeks.

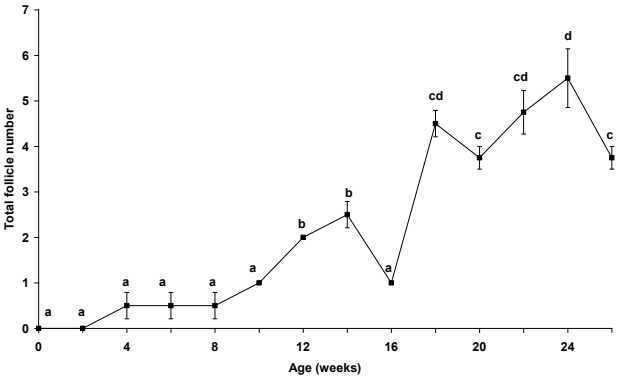

Figure 3: Number of all follicles ≥3mm in diameter in both ovaries determined at different ages in ewe lambs (mean ± SEM, n=4). Four animals were killed at each age. Average values with different letters differ significantly (P<0.05).

The maximal follicular diameter decreased significantly between the birth and one week (P<0.05). However, it rose between 1 week and 4 weeks after birth (P<0.05, figure 4), and between 8 and 10 weeks. It rose again between 16 and 18 weeks and was highest in 24-week-old animals.

The maximal follicular diameter was >3mm (3.55 ± 0.13mm) at 10 weeks, greater than 4mm (4.89 ± 0.25mm) at 18 weeks, and peaked at 24 weeks (7.23 ± 0.16mm) (figure 4).These increases in the maximum diameter of antral follicles corresponded to an increase in plasma concentrations of both FSH and LH at 10 and 18 weeks, and to the preovulatory LH surge at 24 weeks (figure 1). The follicle of the maximum diameter was assumed to represent the preovulatory follicle.

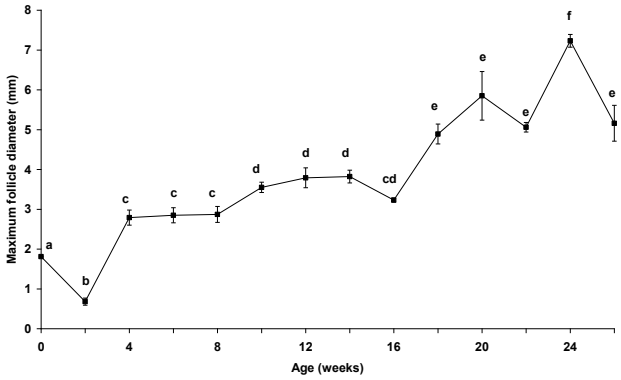

Figure 4: Maximum diameters of all follicles ≥3mm in diameter in both ovaries determined at different ages in ewe lambs (mean ± SEM, n=4). Four animals were killed at each age. Average values with different letters differ significantly (P<0.05).

DISCUSSION

Peripheral plasma FSH and LH increased significantly from birth to 10 weeks of age. After this age, both FSH and LH declined to levels that remained higher than the levels in the neonate and were similar to the levels in adult sheep. This is in agreement with previous reports [7] that found that both FSH and LH increased in Bulgarian sheep from 10 days after birth. However, the average plasma concentrations of FSH and LH in Merino [18, 19] and Shropshire ewe lambs [20] did not vary significantly throughout the first 10 weeks after birth. In another study, a marked increase in serum LH was reported between birth and 18 days [21]. The peak concentration of FSH was recorded at 8 weeks old in Bulgarian sheep [9]. These observations could be explained by physiological differences between breeds.

When we studied the morphological development of ovaries from Ouled Djellel ewe lambs from which blood was collected during the same postnatal period, the average ovarian weight increased significantly from birth to 8 and 10 weeks, in parallel with the variation in plasma levels of gonadotropin hormones. It is important to note that the increase in FSH and LH from 4 weeks to 10 weeks of age can explain the increase in size of antral follicles beyond 2mm. At this size, the tonic growth of follicles is acutely dependent on gonadotrophin support in sheep [7, 22, 1, 23]. However, basal follicular growth can occur in the absence of gonadotropins [24, 25].

In a recent study using ultrasonography in April born Suffolk x Western White Face sheep, an increase in the number of follicles ≥3 mm was observed in 14-week-old animals [26]. In another study, the number of antral follicles in the ovaries of 12-week-old lambs of the Romanov and Ile-de-France breeds was found be higher than that seen in sexually mature animals [27].

Other studies have found abundant vesicular follicles at 4 weeks after birth [28]. None of these studies of ovarian follicular growth was able to explain the observed increase in the number of antral follicles. An increase in FSH concentration has been reported in 8-week-old lambs born from crossbred ewes (East x Black-Head Pleven breeds) x East Friesian rams [9]. However, in another study, circulating FSH remained stable and within the basal levels of the adult cycling female [29].

Other researchers have reported that the average concentration of FSH did not vary during the entire period of sexual development in ewes [30]. However, other workers [31] have reported that FSH increased in 3- to 11-week-old ewe lambs, but that the FSH concentration detected at 11 weeks of age was maintained until the 35-week-old lamb reached puberty. In our results, FSH levels showed only non significant changes from 12 weeks until the age of onset of puberty.

At 10 weeks, we recorded an increase in both FSH and LH. Previous studies in female lambs [21, 32] have indicated that, during the first 6 weeks after birth, daily concentrations of circulating luteinizing hormone were much greater and more variable than baseline levels found in the adult. Other researchers have found that, in 9-week-old animals, an increase in LH [29] to approximately that of adult ewes indicated an adequate pituitary LH level for ovulation [32]. Moreover, the onset of pulsatile LH occurs at about 11 weeks after birth in ewe lambs [33]; at this time; LH levels are similar to those detected at the time of puberty [31].

Other studies have found that LH concentrations are higher at puberty than 7 weeks before puberty in ewe lambs [34], which agrees with the results of this study at 10 weeks of age. This increase in LH plasma concentration is correlated to the presence of follicles ≥2mm in diameter, as stated previously. At this follicular size, the numbers of FSH receptors on the granulosa cells and LH receptors on the thecal layer are maximal [35] and aromatase activity becomes detectable in granulosa cells [36].

The increase in FSH and LH levels at 18 weeks after birth can be explained by the presence of follicles >4mm in diameter. In a recent study, Bartlewski et al [26] found an increase in the number of follicles of ≥3mm at 4 months of age. They did not give an explanation for this increase. It has been observed that LH receptors are expressed on follicles when they reach 4mm in diameter [5]. Previous study has demonstrated that FSH induces LH receptors in granulosa cells [37], functional thecal and granulosa LH

receptors, and the formation of an active aromatase system [38].

The peak of LH plasma levels at 24 weeks, which increased by 9.54-fold over that recorded at the previous age, suggests that the first preovulatory LH surge occurs in most Ouled Djellel ewe lambs at 24 weeks of age. Akchiche [39] found that the preovulatory LH surge was higher than 2ng/ml in adult cyclic Ouled Djellel ewes, and the basal concentration of LH was lower than 1ng/ml. This suggestion is supported by observations of the morphological development of ovaries of the same animals at this age.

Ovaries are characterized by rapid maturation, and the presence of a corpus luteum. It is known that corpora lutea are identifiable with ultrasonography from approximately 3 days after ovulation until the next oestrus in ewes [40, 41]. It is important to note that all the ewes studied here did not reach puberty at the same time. This explains the high standard deviation of LH concentration recorded at 6 months and reflects the differences between animals of the same breed. We recorded the ovulatory LH peak in only one ewe because this LH peak lasts 8 to 10 hours, and our sampling frequency was once every 15 days.

In this study, the average diameter of the preovulatory follicle was 7.23 ± 0.16mm in Ouled Djellel ewe lambs. The maximum diameter reached by preovulatory follicles is higher in non prolific breeds of sheep [27, 42, 43]. A smaller ovulatory follicle size has consistently been reported for prolific breeds such as the Booroola [41], Finn [45, 43], Romanov [27], Booroola x Suffok, and Booroola x Finnish Landrace [42]. Higher ovulation rates are often accompanied by smaller ovulatory follicles [46]. It is known that follicles 4–7mm in diameter are able to ovulate [41, 47] in respond to an LH surge, because granulosa cells within the follicles have LH receptors at this size [48].

Acquisition of LH receptors by granulosa cells is a later sign of functional maturity of the preovulatory follicle. In sheep, the growth of antral follicles is primarily dependent on FSH, and the terminal phase of follicular development, culminating in ovulation, is under the control of LH [49, 50, 51]**.** Acquisition of a fully functional aromatase system and maturation of the central nervous system are required for puberty to proceed. It is well known that the first behavioural oestrus can occur after 1–6 ovulations [52], or after the first LH surge when it is followed by a normal full luteal phase [10]. Therefore full reproductive capacity, i.e. sexual maturity, of ewe lambs is reached earlier, at 26–28 weeks of age, in the Ouled Djellel breed than in other breeds of sheep.

Our results indicate that: 1) there is a distinctive pattern in the variation of concentrations of circulating FSH and LH in Ouled Djellel sheep, which reflects their reproductive capacity, and 2) ewe lambs of this breed can attain puberty as early as 6 months of age. Therefore, sheep of this breed may be fertilized at an early age (12 months) instead of 18 months if they have reached 60–70% of the body weight of adult ewes.

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