

SEASONAL CHANGES IN PLASMA LEVELS OF FSH, LH, AND TESTOSTERONE IN LOCAL RAMS RAISED IN SOUTHWESTERN ALGERIA

Fouad Gani ^{1,*}, Abdellatif Niar ¹

¹ Laboratoire de Reproduction des Animaux de la Ferme, Institut des Sciences Vétérinaires,
Université Ibn Khaldoun de Tiaret, BP 78, Tiaret 14000, Algeria



Abstract

The objective of this work was to investigate the seasonal variations of sexual activity of local rams raised in a desert environment in Southwestern Algeria, by measuring the changes in plasma concentrations of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone. A total of 60 mature and healthy rams of local breeds, mainly Ouled Djellal, belonging to 14 breeders in the area of Béchar and aged 1 to 4 years, were involved to determine the hormonal profiles of FSH, LH, and testosterone during the four seasons of the year (spring, summer, autumn, and winter). Blood samples were collected once or twice each month, with an average of 5 rams. Hormonal assays were measured by using the chemiluminescent immunoenzymatic technique (CLIA). Our results revealed that the changes in the concentrations of all the studied hormones were not statistically significant between months or seasons ($p > 0.05$), in addition to the total absence of a non-breeding season. These allow us to deduce that the effects of seasonality are minor to negligible, and consequently, the local rams seem to have a continuous sexual activity all year around in Southwestern Algeria.

Keywords: desert, hormonal profile, Ouled Djellal, seasonal variation, sexual activity

1. INTRODUCTION

It is well known that the sheep breeds originating from temperate climates in mid or high latitudes display marked seasonal variation in reproductive activity. On the other hand, in tropical and subtropical environments, the ewes are either completely aseasonal or intermittently polyoestrus with the quality and availability of food dictating breeding activity (Rosa and Bryant, 2003). The rams also show seasonal changes in reproduction including sexual behavior, semen quality, hormonal activity, gametogenesis, and testicular size (Dufour et al., 1984; Mandiki et al., 1998; Langford et al., 1999; Santos et al., 2015).

Previous studies have successfully used circulating reproductive hormones (FSH, LH, and testosterone) to identify the reproductive performance and the potential of rams (Sanford et al., 1977; Dickson and Sanford, 2005; Kishk, 2008). Besides, FSH acts specifically on Sertoli cells, playing an important role in the maintenance of qualitatively and quantitatively normal spermatogenesis (Nieschlag et al., 1999). LH acts on Leydig cells to stimulate the testosterone secretion (Schlatt and Ehmcke, 2014). The testosterone secretion, that is the major testicular androgen, is a necessary prerequisite for the maintenance of established spermatogenesis in the adult testes (Zirkin, 1998).

Furthermore, the variations in sexual activity result from changes in the secretion of these hormones (Karsch et al., 1984; Ortavant et al., 1988; Langford et al., 1998). Under the natural conditions, the levels of these hormones in sheep are influenced by several factors including season through photoperiod, nutrition, genotype, age, geographical location, and social interaction (Sanford et al., 1982; D'occhio et al. 1984; Martin et al., 1994).

In Algeria, sheep farming occupies a very important place in the field of animal production. It is largely dominated by hardy local breeds, spread over several regions of the country. The objective of this work was to investigate the seasonal variations of the sexual activity of rams of our local breeds, raised in a desert environment in Southwestern Algeria, by measuring changes during the different seasons of the year in plasma concentrations of FSH, LH, and testosterone.

2. MATERIALS AND METHODS

Geographical situation and climate

The present experiment was carried out in Southwestern Algeria, in the region of Béchar, at sheep farms located between 30°15'N to 31°03'N latitude and 2°00'W to 2°39'W longitude. This region is characterized by a continental desert climate, with hot summers and cold winters. The precipitations are irregular and low with about 90 mm/year. The day length varies from 9 hours 56 minutes in December to 14 hours 4 minutes in June. This study was conducted over a period of 12 months, from March 2018 to March 2019, during the four seasons: spring (20 March - 21 June), summer (21 June - 23 September), autumn (23 September - 21 December), and winter (21 December - 20 March).

Animals

A total of 60 mature and healthy rams of local breeds, predominately Ouled Djellal and aged 1 to 4 years, were involved in this study. The rams were owned by 14 breeders. In the majority of the herds, the feeding systems were mainly based on the use of natural pastures throughout the day with a concentrated feed supplement which varied from 200 to 500 g per head and per day, depending on the actual status of the pastures. The other herds were kept in permanent housing, with no access to pasture during all over the year, and fed rations made up of hay, concentrates, and on-farm crop residues. In either case, the animals were maintained under conditions of natural lighting.

Blood samples and hormonal assays

The blood samples were taken once or twice a month, averaging 5.0 ± 2.0 rams per month in the jugular vein, using the Vacutainer system on heparinized lithium tubes during the four seasons of the year. After centrifugation at 2875 rpm for 15 minutes, the plasma was isolated and stored at -20 °C until analysis. FSH, LH and testosterone were determined with chemiluminescent immunoenzymatic method (Rojanasakul et al., 1994; Ayad et al., 2018) using the Access Immunoassay Systems on a Beckman coulter automate.

Statistical analysis

The serum FSH, LH, and testosterone concentrations were subjected to a one-way analysis of variance (ANOVA) to compare the hormone levels between seasonal and monthly data sets, with values of $p < 0.05$ were considered as statistically significant. The correlations among hormones for each season were evaluated by a simple Pearson correlation..

3. RESULTS AND DISCUSSIONS

Hormonal profiles

The data on the reproductive endocrine status demonstrated that the hormonal profiles of FSH, LH, and testosterone exhibited slight changes between some months and seasons (Figure 1, Table 1), but in any case, the differences were not statically significant ($p > 0.05$).

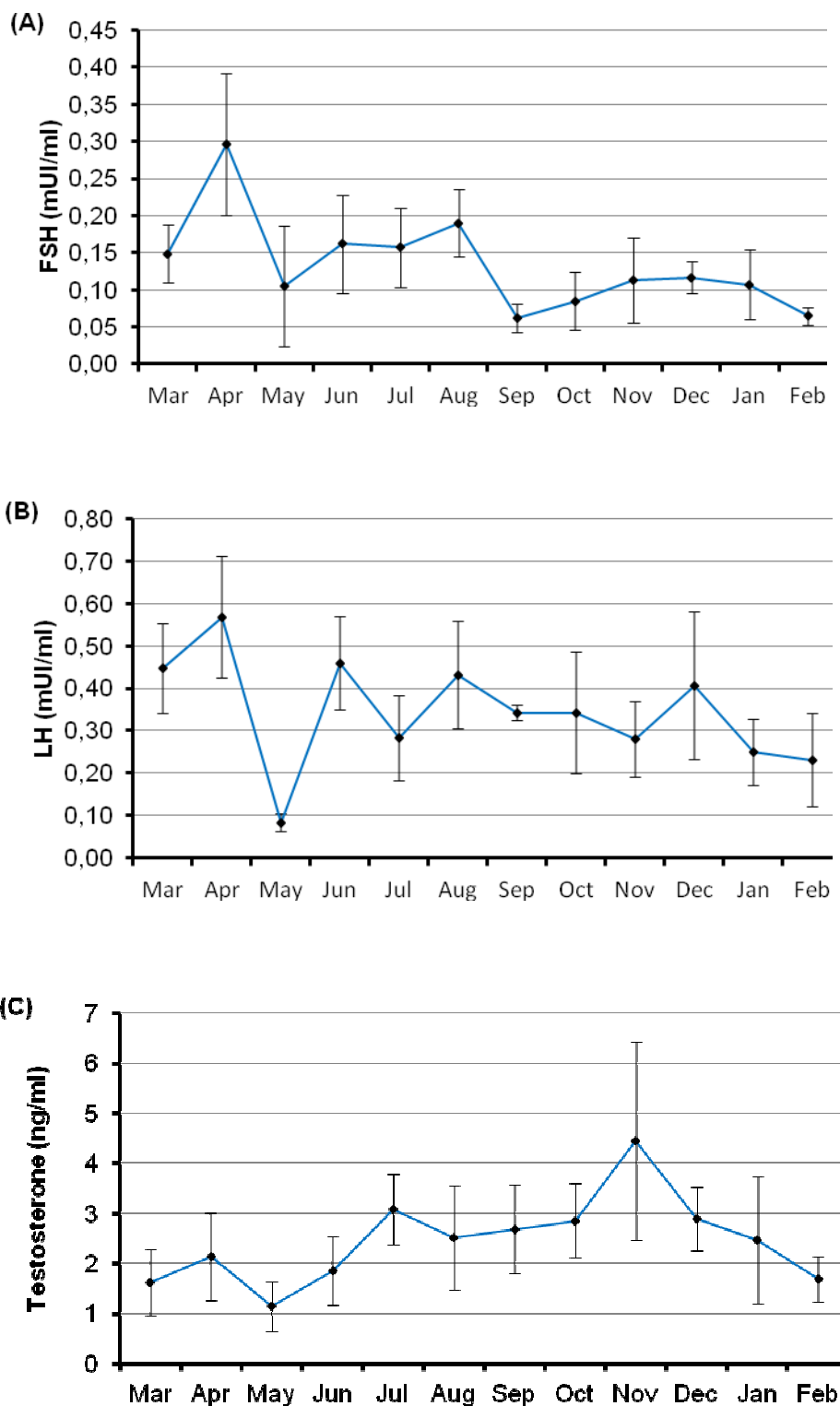


Figure 1. Monthly plasma concentrations (mean \pm S.E.M.) of FSH (A), LH (B), and testosterone (C) hormones in rams ($n = 5 \pm 2$ for each month)

Table 1. Plasma concentrations (mean \pm SEM) of FSH, LH, and testosterone in rams according to seasons

Hormone	Saison			
	Spring (n = 13)	Summer (n = 17)	Autumn (n = 15)	Winter (n = 15)
FSH (mUI/mL)	0.21 \pm 0.06	0.17 \pm 0.03	0.09 \pm 0.02	0.10 \pm 0.02
LH (mUI/mL)	0.39 \pm 0.10	0.38 \pm 0.06	0.34 \pm 0.05	0.32 \pm 0.07
Testosterone (ng/mL)	1.68 \pm 0.51	2.55 \pm 0.46	3.23 \pm 0.59	1.88 \pm 0.41

The maximum plasma FSH concentration was recorded in April, with a value of about 0.30 ± 0.09 mIU/mL, then it decreased in May, and then the concentrations appeared to be very close during June to March. The average values of seasons ranged from 0.09 ± 0.02 mUI/mL in autumn to 0.21 ± 0.06 mUI/mL in spring.

Similarly, the plasma concentration of LH was maximum in April (0.57 ± 0.14 mIU/mL), then it decreased significantly in May to reach the value of 0.08 ± 0.02 mIU/mL. From June to March, the LH levels were relatively constant (0.23 to 0.46 mIU/mL). In fact, the average values of all seasons were very close.

For testosterone, the concentration gradually increased from May to peak in November (4.44 ± 1.99 ng/mL), then it declined to reach for 1.68 ± 0.45 ng/ml in February. After that, the contents took values close until April. In general, the average concentrations varied from 1.68 ± 0.51 ng/mL in spring to 3.23 ± 0.59 ng/mL in autumn.

It is known that the male sexual activity depends on the pituitary gonadotrophic function (Ortavant et al., 1988). Also, the release of FSH and LH from pituitary gonadotropins is controlled by GnRH. This master switch can use the photoperiod information to control the activity of the reproductive organs in synchrony with the seasonal changes (Schlatt and Ehmcke, 2014). To this end, mostly it has been established that the secretion of FSH and LH was higher during the breeding season than during the non-breeding season (Tilbrook et al., 1999; Hafez ESE and Hafez B., 2000). In this context, Thimonier (1981) has reported that FSH and LH concentrations in the pituitary are reduced to 50% during the non-breeding season. Therefore, in this study, the monitoring of hormonal profiles of FSH and LH indicate the absence of non-breeding season throughout the year.

By contrast, several studies have found significant difference between the seasons in the hormonal profiles of these two hormones. As regards FSH, some researchers (Sanford et al., 1976; Kennaway et al., 1981) have demonstrated a rise in the level of plasma FSH in Finnish Landrace rams in Canada and in Saxon Merino rams from April to May with a maximum in August-September followed by a drop. Barrell et al. (1987) have found that New Zealand Romney rams on pasture have a seasonal pattern of plasma FSH levels with a peak from January to March. Concerning LH, Schanbacher and Lunstra (1976), according to their study with Finnish Landrace and Suffolk rams in Nebraska (USA), have noted that serum LH concentrations were lowest in May and increased abruptly in July when daily photoperiod began to decrease. Similarly, Misztal et al. (1996) have found that the lowest mean concentrations of LH occurred in rams of Polish lowland breed during the period of the longest day from June to the end of July.

In addition, in the present study, the maximum average concentration of testosterone is recorded in autumn, but generally without significant difference between months or seasons. In accordance to our results, Belkhir et al. (2017) have found no differences between seasons in their experiments with Ouled Djellal rams in the East of Algeria. The same result is obtained by Fernandez-Abella et al. (1999) with Merino and Corriedale breeds in Uruguay.

In contrast, several studies have reported that the serum testosterone concentration varied with season of the year. Some authors (Schanbacher and Lunstra 1976; Misztal et al., 1996; Zamiri and Khodaei, 2005; Casao et al., 2010; Zamiri et al., 2010) have showed that serum testosterone level was lowest from April to July and increased thereafter reaching highest values in October - November (autumn) in each of Finnish Landrace and Suffolk in Nebraska, Polish Lowland, Iranian Ghezel, Mehraban and Moghani rams, and Rasa Aragonesa rams in Zaragoza, whereas others authors (Aller et al., 2012; Milczewski et al., 2015; Ahmad et al., 2018) have found the highest level in summer for Argentine Pampinta and Corriedale rams, Suffolk ram in the south of Brazil, and Kivircik rams in Aydin (Turkey).

In this respect, Gani and Niar (2019) have found that Ouled Djellal ewes raised under difficult climatic conditions of the south-west of Algeria are relatively non-seasonal with sometimes only a slight decrease in reproductive activity. Also, in the region of Chlef, the Ouled Djellal ewe presents a sexual activity all year round, even during the light seasonal anoestrus (Taherti et al., 2016; Zidane and Ababou, 2017). This allows us to support the character of the ability to breed off-season in Ouled Djellal breed.

Correlations between hormones

The relationship between FSH, LH, and testosterone at each season of the year are presented in Table 2.

Table 2. Pearson's correlations (r) between FSH, LH and testosterone per season in ram seminal plasma.

Variables	Saison				
	Spring	Summer	Autumn	Winter	
FSH (mUI/mL) – LH (mUI/mL)	r	0,81**	0,57*	0,64*	0,79**
	sig	0,001	0,02	0,01	0,00
Testosterone (ng/mL) – FSH (mUI/mL)	r	-0,06	-0,25	-0,34	0,12
	sig	0,84	0,33	0,22	0,67
Testosterone (ng/mL) – LH (mUI/mL)	r	-0,04	-0,34	-0,34	0,35
	sig	0,89	0,18	0,22	0,20

*. The correlation is significant at the 0.05 level.

**.. The correlation is significant at the 0.01 level.

The Table 2 shows that the serum FSH levels were significantly correlated with the serum LH levels during all seasons. This indicates a direct relationship between the two variables over the year. Likewise, Lincoln and Davidson (1977) have obtained a parallel variation in the levels of both FSH and LH in adult Soay rams in Scotland.

Also, the Table 2 shows that the correlations testosterone – FSH and testosterone – LH were not statistically significant in all seasons. Tilbrook et al. (1999) have noticed that the testosterone acts on the pituitary gland to suppress the secretion of FSH, and these responses are affected by the season. Testosterone regulates the FSH secretion during the non breeding season, but not during the breeding season. Also, Rhim et al. (1993) have claimed that in Hampshire rams in Urbana (USA), testosterone was significantly correlated to LH in the transition period from breeding to non breeding season and the non breeding season but not in the breeding season. Therefore, this can be

justified by the absence of non breeding season, according to the results of correlations obtained in this study.

4. CONCLUSIONS

The results obtained indicate that the effects of seasonality are minor to negligible, and consequently local rams seem to have a sexual activity all year around in south-west of Algeria. Further studies are needed to go deeper on the reproductive performance of rams raised in this region, including other examinations such as semen examination, scrotal measurement, and libido testing

5. ACKNOWLEDGEMENTS

The authors sincerely thank all the local breeders who generously offered their animals, for the time spent during sampling, and for their support during this study.

6. REFERENCES

- Ahmad, E., Naseer, Z., Uçan, U., Serin, İ., Ceylan, A., Aksoy, M. (2018). Seasonal variations in sperm acrosome reaction, osmotic tolerance and serum testosterone concentrations in rams. *Animal reproduction science*, 198, 112-120.
- Aller, J.F., Aguilar, D., Vera, T., Almeida, G. P., Alberio, R. H. (2012). Seasonal variation in sexual behavior, plasma testosterone and semen characteristics of Argentine Pampinta and Corriedale rams. *Spanish journal of agricultural research*, 10(2), 345-352.
- Ayad, A. Benhanifia, M. and Benbarek, H. (2018). The ability of human electrochemiluminescence immunoassay to measure testosterone and progesterone in ovine plasma. *Malaysian Journal of Veterinary Research*, 9(1), 22-30.
- Barrell, G. K., Lapwood, K. R., Elgar, H. J. (1987). Seasonality of secretion of follicle stimulating hormone in New Zealand Romney rams and effects of cranial sympathetic ganglionectomy. *New Zealand Journal of Agricultural Research*, 30(4), 439-442.
- Belkhiri, Y., Bouzebda-Afri, F., Bouzebda, Z., Mouffok, C., Djaout, A. (2017). Seasonal variations in reproductive parameters of Ouled Djellal rams in the East of Algeria. *Indian Journal of Animal Research*. DOI: 10.18805/ijar.v0i0F.7266
- Casao, A.; Cebrián, I.; Asumpção, M. E.; Pérez-pé, R.; Abecia, J. A.; Forcada, F.; Cebrián-Pérez, A. J. and Muñoz-Blanco, T. (2010). Seasonal variations of melatonin in ram seminal plasma are correlated to those of testosterone and antioxidant enzymes. *Reproductive biology and endocrinology*, 8(1), 59.
- D'occhio, M. J., Schanbacher, B. D., Kinder, J. E. (1984). Profiles of luteinizing hormone, follicle-stimulating hormone, testosterone and prolactin in rams of diverse breeds: effects of contrasting short (8L: 16D) and long (16L: 8D) photoperiods. *Biology of reproduction*, 30(5), 1039-1054.
- Dickson, K. A. and Sanford, L. M. (2005). Breed diversity in FSH, LH and testosterone regulation of testicular function and in libido of young adult rams on the Southeastern Canadian prairies. *Small ruminant research*, 56(1-3), 189-203.
- Dufour, J. J.; Fahmy, M. H. and Minvielle, F. (1984). Seasonal changes in breeding activity, testicular size, testosterone concentration and seminal characteristics in rams with long or short breeding season. *Journal of animal science*, 58(2), 416-422.
- Fernandez-Abella, D., Becu-Villalobos, D., Lacau-Mengido, I. M., Villegas, N. Bentancur, O. (1999). Sperm production, testicular size, serum gonadotropins and testosterone levels in Merino and Corriedale breeds. *Reproduction nutrition development*, 39(5-6), 617-624.
- Gani, F., Niar, A. (2019). Seasonal variations of the reproductive activity in local breed's ewes raised in the south-west of Algeria. *Bionature*, 39(2), 116-124.
- Hafez, E.S.E., Hafez, B. (2000). Reproductive Cycles. In: E.S.E. Hafez and B. Hafez Eds, *Reproduction in Farm Animals* (7th edition. pp. 55-67). Lippincott Williams and Wilkins, South Carolina, USA.
- Karsch, F. J., Bittman, E. L., Foster, D. L., Goodman, R. L.; Legan, S. J., Robinson, J. E. (1984). Neuroendocrine basis of seasonal reproduction. In *Proceedings of the 1983 Laurentian Hormone Conference* (pp. 185-232). Academic Press.
- Kennaway, D. J., Obst, J. M., Dunstan, E. A., Friesen, H. G. (1981). Ultradian and seasonal rhythms in plasma gonadotropins, prolactin, cortisol, and testosterone in pinealectomized rams. *Endocrinology*, 108(2), 639-646.

- Kishk, W. H. (2008). Interrelationship between ram plasma testosterone level and some semen characteristics. *Slovak journal of animal science*, 41(2), 67-71.
- Langford, G. A., Sanford, L. M., Marcus, G. J., Shrestha, J. N. B. (1999). Seasonal cyclic pituitary and testicular activities in rams. *Small Ruminant Research*, 33(1), 43-53.
- Langford, G. A., Shrestha, J. N. B., Sanford, L. M., Marcus, G. J. (1998). Reproductive hormone levels of early postpubertal ram lambs in relation to breed, adult testis size and semen quality. *Small Ruminant Research*, 29(2), 225-231.
- Lincoln, Ga., Davidson, W. (1977). The relationship between sexual and aggressive behaviour, and pituitary and testicular activity during the seasonal sexual cycle of rams, and the influence of photoperiod. *Reproduction*, 49(2), 267-276.
- Mandiki, S. N. M., Derycke, G., Bister, J. L., Paquay, R. (1998). Influence of season and age on sexual maturation parameters in Texel, Suffolk and Ile-de-France rams: 2. Circulating concentrations of follicle stimulating hormone, luteinizing hormone, prolactin and testosterone. *Small ruminant research*, 28(1), 81-88.
- Martin, G B, Walkden-Brown, S W, Boukhliq, R, Tjondronegoro, S, Miller, D. W., Fisher, J. S., Hötzel, M. J., Restall, B. J., Adams, N. R. (1994). Non-photoperiodic inputs into seasonal breeding in male ruminants. *Perspectives in comparative endocrinology*, 574-585.
- Milczewski, V., Chahad-Ehlers, S., Spencoski, K. M., Morais, R. N., Soccol, V. T. (2015). Quantifying the effect of seasonality on testicular function of Suffolk ram in lower latitude. *Small Ruminant Research*, 124, 68-75.
- Misztal, T., Romanowicz, K., Barcikowski, B. (1996). Seasonal changes of melatonin secretion in relation to the reproductive cycle in sheep. *J Anim Feed Sci*, 5, 35-48.
- Nieschlag, E., Simoni, M., Gromoll, J., Weinbauer, G. F. (1999). Role of FSH in the regulation of spermatogenesis: clinical aspects. *Clinical endocrinology*, 51(2), 139-146.
- Ortavant, R., Bocquier, F., Pelletier, J., Ravault, J. P., Thimonier, J., Volland-Nail, P. (1988). Seasonality of reproduction in sheep and its control by photoperiod. *Australian journal of biological sciences*, 41(1), 69-86.
- Rhim T, J., Kuehl, D., Jackson, G. L. (1993). Seasonal changes in the relationships between secretion of gonadotropin-releasing hormone, luteinizing hormone, and testosterone in the ram. *Biology of reproduction*, 48(1), 197-204.
- Rojanasakul, A., Udomsuppayakul, U., Chinsomboon, S. (1994). Chemiluminescence immunoassay versus radioimmunoassay for the measurement of reproductive hormones. *International Journal of Gynecology & Obstetrics*, 45(2), 141-146.
- Rosa, H J D., Bryant, M. J. (2003). Seasonality of reproduction in sheep. *Small ruminant research*, 48(3), 155-171.
- Sanford, L. M., Howland, B., Faiman, C., Palmer, W. M. (1976). The profile of follicle-stimulating hormone secretion in the ram. *Canadian Journal of Animal Science*, 56(3), 497-504.
- Sanford, L. M., Palmer, W. M., Howland, B. E. (1977). Changes in the profiles of serum LH, FSH and testosterone, and in mating performance and ejaculate volume in the ram during the ovine breeding season. *Journal of animal science*, 45(6), 1382-1391.
- Sanford, L. M., Palmer, W. M., Howland, B. E. (1982). Influence of age and breed on circulating LH, FSH and testosterone levels in the ram. *Canadian Journal of Animal Science*, 62(3), 767-776.
- Santos, S. I., Sánchez-Dávila, F., Vázquez-Armijo, J. F., Ledezma-Torres, R. A., Del Bosque-González, A. S., Palomera, C. L., Bernal-Barragán, H. (2015). Changes in sexual behaviour and semen quality associated with age and type of enclosure of Saint Croix rams in different seasons of the year. *Italian Journal of Animal Science*, 14(4), 3890.
- Schanbacher, B. D., Lunstra, D. D. (1976). Seasonal changes in sexual activity and serum levels of LH and testosterone in Finish Landrace and Suffolk rams. *Journal of animal science*, 43(3), 644-650.
- Schlatt, S. and Ehmccke, J. (2014). Regulation of spermatogenesis: an evolutionary biologist's perspective. *In Seminars in cell & developmental biology* (Vol. 29, pp. 2-16). Academic Press.
- Taherti, M.; Kaidi, R., Aggad, H. (2016). Variations mensuelles de l'activité sexuelle de la brebis Ouled Djellal élevée dans la région de Chlef, Algérie. *Livestock Research for Rural Development*. 28 (1).
- Thimonier, J. (1981). Control of seasonal reproduction in sheep and goats by light and hormones. *Journal of reproduction and fertility*. Supplement, 30, 33-45.
- Tilbrook, A. J., De Kretser, D. M., Clarke, I. J. (1999). Seasonal changes in the negative feedback regulation of the secretion of the gonadotrophins by testosterone and inhibin in rams. *Journal of endocrinology*, 160(1), 155.
- Zamiri, M. J., Khodaei, H. R. (2005). Seasonal thyroidal activity and reproductive characteristics of Iranian fat-tailed rams. *Animal Reproduction Science*, 88(3-4), 245-255.
- Zamiri, M J., Khalili, B., Jafaroghli, M., Farshad, A. (2010). Seasonal variation in seminal parameters, testicular size, and plasma testosterone concentration in Iranian Moghani rams. *Small Ruminant Research*, 94(1-3), 132-136.

- Zidane, A., Ababou, A. (2017). Variations hormonales saisonnières de brebis Ouled Djellal dans la région de Chlef, Algérie. *Livestock Research for Rural Development*. 29(12).
- Zirkin, B. R. (1998). Spermatogenesis: its regulation by testosterone and FSH. In *Seminars in cell & developmental biology* (Vol. 9, No. 4, pp. 417-421). Academic Press.