https://iphopen.org/index.php/
Online ISSN: 3050-8835 Print ISSN: 3050-9289

HORMONAL INFLUENCE ON OOCYTE DEVELOPMENT: AN EVALUATION OF LH AND FSH EFFECTS IN AWASSI AND HAMDANI SHEEP BREEDS

Mohammed Hatem Abed*

*Department of Pathological Analysis, College of Applied Medical Sciences, Al-Muthanna University,Al-rumaytha 66002, Iraq.

***Corresponding Author: Mohammed Hatem Abed**

ABSTRACT

This investigation aimed to assess the effects of circulating follicle stimulating hormone (FSH) and luteinizing hormone (LH) levels on oocyte maturity in sheep of the indigenous Awassi and Hamdani breeds. Oocytes were obtained from 48 mature females and evenly distributed between the two breeds for experimental use. The same reproductive synchronization protocol was used for both breeds which included the insertion of a P4 (progesterone)-loaded intravaginal device followed by injecting pregnant mare serum gonadotropin (PMSG) after removal of the device. Blood samples for analysis of circulating gonadotropin levels (measured by ELISA) were collected from the jugular vein 48 hours after removal of the P4. Oocyte maturity was assessed morphologically using conventional light microscopic techniques. Statistical analysis of the data demonstrated significant differences between breeds in regard to oocyte maturity. The Awassi breed exhibited significantly higher levels of LH (0.41 ± 0.36) as well as a greater number of developmentally competent oocytes (76.48 ± 1.33) and fewer degenerative oocytes (10.29 ± 1.41) and immature gametes (13.38 ± 0.38) than the Hamdani breed. Additionally, the FSH (0.53 ± 0.19) levels of the Awassi were significantly higher than the Hamdani. Therefore, it can be concluded that gonadotropic hormones are important markers of oocyte developmental potential and that the Awassi breed has greater hormonal regulation and reproductive potential than the Hamdani breed. This data supports the concept that specific_ breed hormonal protocols stimulation could greatly enhance the success rate of assisted reproductive technologies and genetically selective breeding programs utilizing indigenous ovine genetic resources.

Keywords: Artificial Insemination, LH, FSH, Awassi Sheep, Oocyte Maturation, Hamdani Sheep

DOI:-10.5281/zenodo.18618321 **Manu script # 401**

Introduction

In ruminants, there are highly organized reproductive systems that rely on hormones for different stages of developing eggs, cells maturing and Oocytes 's being released from the ovary. The hormone systems have two parts: One part is the anterior pituitary gland, which produces the gonadotropins, such as follicle stimulating hormone (FSH) and luteinizing hormone (LH). These two hormones are critical in establishing a follicle's (Oocytes 's) development, and they also help in the establishment of the Oocytes 's overall development as it matured into an Oocytes 's (1,2,3). FSH acts on the follicles by stimulating the growth and development of the ovarian granulosa cells of the developing follicles, causing the cells to reproduce and grow within the follicles, and causing production of estrogen within these cells. The establishment of estrogen affects the cellular environment of the follicles and provides conditions that allow oocyte maturation (4,5). On the other hand, (LH) acts on the theca cells of the follicles, producing androgens, and as a result will trigger the onset of ovulation and form the corpus luteum (6,7).

The Awassi and Hamdani breeds of sheep are genetically different breeds that are both economically viable in the Iraqi livestock industry, and they exhibit unique issues related to their reproductive physiology and respective production capabilities. While several studies show that Awassi ewes are reasonably well suited to the arid climate conditions of Iraq, they show moderate reproductive success, whereas the Hamdani breed has much higher reproductive success and a lot of ability to tolerate extreme environmental conditions and would thus be very appropriate as a representative animal model for any future studies using applied reproductive biology techniques (8,9). Studies have shown a considerable positive correlation between circulating gonadotropin levels and embryo growth as well as general reproductive success (10,11). Additionally, there appear to be significant genetic differences effectively among the two breeds regarding the ovarian tissue responsiveness to gonadotropin stimulation (12,5), providing evidence for the importance of comparing applied reproductive biology between the two breeds. IVM techniques will allow researchers to determine the estrogenic profile as well as to assess the degree to which each of the two breeds is capable of promoting oocyte maturation in vitro and to compare each of the breeds' respective responsiveness to gonadotropin stimulation (13). Therefore, this study's aim is to compare, the circulating levels of (LH) and (FSH) in the two breeds and compare how each of those hormones influences the rate of maturation of oocytes from ewes in each breed. Ultimately, this will contribute to the optimization of key aspects of artificial reproductive techniques, as well as to assist with the genetic selection of the most productive sheep, and thus build upon the existing knowledge base in reproductive performance based on genetic differences in breeding.

Materials and Methods

Experimental Animals

This study utilized 48 female sheep that were reproductive young adults from 2 breeds, Awassi (n=24) and Hamdani (n=24) and all animals were approximately 2.5-4 years old, weighing an average of 48 ± 7 kg. The selected animals were from Indigenous flocks located in Al-Muthanna Province, Iraq. Each animal was evaluated prior to beginning the experiment by a licensed veterinarian to determine; The animals were clinically healthy and there were no pregnant females, and each sheep appeared to be physiologically appropriate. The sheep received two different types of feed during the experimental period; Concentrated/processed supplemental feeds and fresh forage that were both designed to meet the energy, and protein needs required per (NRC 2007) (14) recommendations. Reproductive synchrony was achieved through use of intravaginal, controlled-release progesterone devices [Chronogest®], each containing 20mg of synthetic progestogen. The progestogen devices were retained for 12 days. At day 12, each sheep received one intramuscular(inj) injection of 500 international units of Pregnant Mare Serum Gonadotropin[PMSG: Folligon®] to induce follicular maturation and ovulation (10,4).

Serum Preparation and Blood Collection for Hormonal Assay

An established jugular vein was punctured to obtain venous blood samples. Each experimental subject had their own blood sample at 48 hours post removal of the device. A ten-milliliter sterile syringe was used to collect blood, which was placed immediately into a serum separator tube that contained a clot-promoting gel matrix. Following natural coagulation of the blood, the samples were subjected to centrifugal separation for 15 minutes at 3000 revolutions per minute to separate the serum. The supernatant fraction of the serum was aseptically transferred to sterile containers and frozen at -20°C prior to the performance of biochemical hormonal quantification procedures (15,16).

Hormonal Level Determination

Quantitative measurement of serum gonadotropin levels was accomplished through enzyme-linked immunosorbent assay (ELISA) methodology. Species-specific immunoassay kits (MyBioSource, USA) were

used according to the manufacture's specifications for ovine samples. Spectrophotometric measurements of optical density were taken at a wavelength of 450 nm using a microplate-reader system (17).

Oocyte Collection and Evaluation

The euthanasia of experimental animals following the completion of the insemination procedure was performed at a time interval of thirty-six hours after insemination in compliance with institutional regulations regarding the humane treatment of animals. Ovarian tissues were immediately removed from experimental animals and transported to the facility where studies would be conducted, using a temperature controlled (thirty-seven degrees Celsius) isotonic saline solution supplemented with antimicrobial agents to maintain sterility. Follicular structures were removed during surgical dissection with sterile scalpel instruments and under stereomicroscopic visualization to free oocytes. Oocytes were evaluated for morphology using a light microscope and only those oocytes that appeared morphologically normal and viable were included for analysis; oocytes that exhibited signs of degeneration, were non-viable or had abnormal structures were not used for any of the analyses performed.

Oocyte Classification

After removal from the isolation, oocytes were washed three times in the RBM–1640 medium and placed into sterile petri dishes for the classification of morphology (according to the classification system created by (18)

Viability Assessment

Before oocyte maturation through in vitro procedures, oocyte viability status must be established, as determining cellular viability is a prerequisite for further processing. In order to differentiate viable gametes from non-viable gametes, the vital dye-exclusion methodology using the Trypan Blue dye was utilized. Oocytes that became stained with Trypan Blue and that exhibited strong blue coloration were classified as non-viable; oocytes that did not stain with Trypan Blue were classified as viable (19,20).

In Vitro Maturation Procedure

To evaluate oocyte developmental competence after a 24-hour incubation, oocytes that were previously washed three(3)-times in RBMI-1640 culture medium were placed into multi-well culture plates (four-compartment plates) containing RBMI-1640 medium with 5 IU/mL Equine Chorionic Gonadotropin (eCG), 10 IU/mL Human Chorionic Gonadotropin (hCG), and 1 µg/mL Estradiol. 4–6 oocytes were placed in each of the wells of the four-compartment plates containing standard RBMI-1640 culture medium; then, the wells were overlaid with liquid paraffin to prevent evaporation of the RBMI-1640 culture medium during the incubation of the oocytes. The culture plates containing oocytes were placed in a controlled-atmosphere incubator set at 38.5ºC, 95% relative humidity, and 5% carbon dioxide, for 24 h. After the incubation, oocytes were microscopically evaluated to determine which of the oocytes were competent to develop (17,21) and which oocytes were not competent to develop (17,21).

Statistical Analysis

Statistical analysis of data was performed with SAS (22). Interbreeds comparisons of gonadotropin concentrations and oocyte maturation parameters were performed using an independent samples t-test. The Pearson correlation coefficient method was used to assess the correlation between hormonal concentrations and oocyte developmental status. Significance was accepted at $P \leq 0.05$.

Results and Discussion

Levels of LH and FSH Hormones

Figure 1 shows the rise in the mean concentrations of the hormone luteinizing hormone from blood samples collected from the Awassi breed (0.41 ± 0.36) compared with Hamdani breed (0.19 ± 0.25), indicating that statistically significant differences exist ($P \leq 0.05$) between these two breeds of animal.

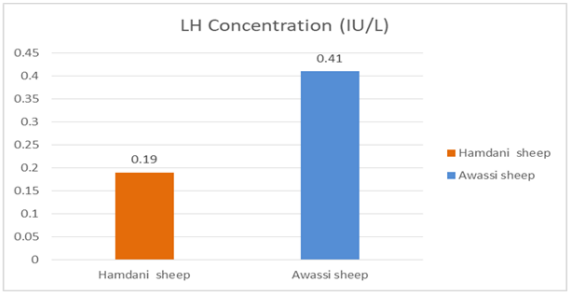

Figure 1. *LH levels in Awassi vs. Hamdani Sheep*

The variations in FSH concentrations in poetry in a given breed and period were also statistically significant, at $P < 0.05$ (cited above). Higher average FSH concentrations (0.53 ± 0.19) were observed in Awassi animals, compared to Hamdani animals (0.32 ± 1.22); an example of this is included in figure 2.

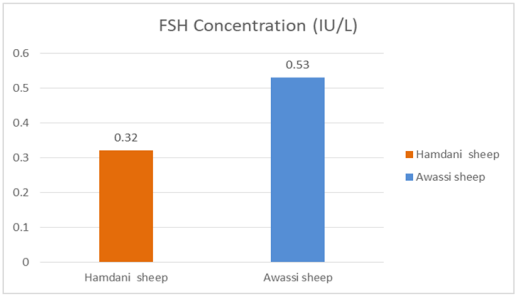

Figure 2. *FSH levels in Awassi vs. Hamdani Sheep*

The enhancement of Awassi reproductive system compared to Hamdani can largely be attributed to many generations of genetic selection that have taken place over many decades, specifically to enhance reproductive efficiency and improve milk production. The genetic selection history documented for Awassi and other breeds is associated with an enhancement of the function of the HPG (hypothalamus–pituitary–gonadal) axis, mainly by the upregulation of gene expression that produces (LH) and (FSH) and also increasing the number (density) of gonadotropin-releasing hormone receptors located in the hypothalamic neutrons. These genetic changes have resulted in both the improvement of pituitary gland sensitivity and the increase of the amount of reproductive hormones secreted (10).

Awassi sheep's higher density of gonadotropin-releasing hormone receptors and their greater sensitivity to gonadotropin-releasing hormone results in the greater LH and FSH secretory response in Awassi when stimulated with endogenous gonadotropin-releasing hormone (23). In addition, the transcription of LH and FSH genes is many times greater in pituitary tissue from Awassi compared to term pituitary tissue from Hamdani sheep (24).

Another contributing factor to the superior reproductive performance in Awassi sheep is the expression of enhanced nutritional and environmental responsiveness. Research has demonstrated that Awassi sheep exhibit enhanced positive physiological response to protein and mineral-supplemented diets, which increases pituitary responsiveness. These observations parallel the more efficient regulation of leptin and insulin-like growth factor 1 (IGF-1), which are both critical metabolic regulators that stimulate gonadotropin-releasing hormone secretion and ultimately drive the production of LH and FSH (25).

Awassi sheep are less affected by the length of the day during their reproductive cycle and can reproduce throughout the year, allowing for consistent levels of gonadotropin at all times during the year. The fact that they have a relatively high baseline level of gonadotropin during all times of the year and still exhibit high levels of gonadotropin during periods without reproductive activity may indicate that this breed has higher levels of physiological plasticity related to the regulation of the reproductive axis and thus, reproductive advantages (26).

Percentage of Mature, Degenerated, and Immature Oocytes in Relation to the Presence or Absence of Cumulus Oophorus

The oocytes were separated into two groups based on whether they had cumulus oophorus present (with or without cumulus cell support) or not have an association with cumulus cells (again, with or without cumulus cells). The data of cumulus-oocyte development are shown in **Figure 3** where the cumulus-oocyte development of the Awassi breed was represented by significantly better developmental competency ($P \leq 0.05$) than that of the Hamdani breed.

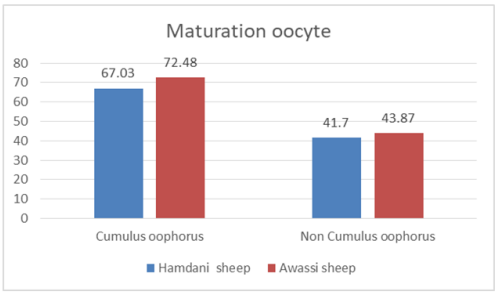

Figure 3. *Percentage of mature Oocytes with/respect to Cumulus Oophorus within Awassi vs. Hamdani Sheep*

The rate of development of Awassi oocytes which have no cumulus cells', ie. oocytes from animals of either species, is found to develop faster than the rate of development of Hamdani oocytes. It is believed that this increased rate of development is due to the beneficial characteristics of the Awassi breed that enhance their ability to provide efficient oocyte maturation. Specifically, Awassi animals are believed to have several biological advantages Dr., such as being more responsive to gonadotropic hormones than Hamdani animals, which facilitates an increase in the rate of development of oocytes which are able to develop (10).

Furthermore, the oocyte development of both Awassi and Hamdani animals is greatly affected by their respective genetics and selective breeding (A), (26). Therefore, the overall performance of Awassi / Hamdani animal reproduction is influenced by the greater accessibility of the ovaries of Awassi animals, as evidenced by the number of follicles produced by Awassi animals that become developed (to maturity), relative to Hamdani animals.

Cumulus cells surrounding the oocyte are equally important to the oocyte development, during the oocyte maturation phase, respectively. There is evidence that the cumulus cells of Awassi animals are better than cumulus cells of Hamdani in their role of developing communications from other cells, and providing nutritional and endocrine support, as in the case of the oocyte secretory factors(GDF9) and (BMP15) , throughout the oocyte maturation period (27).

In addition to genetic and somatic cells, an animal's environment, as well as its nutrition, influences their oocyte maturation. In this regard, Awassi animals have a greater potential to adapt to artificial herding and management systems than do Hamdani animals, resulting in enhanced availability of nutrients and limiting the degree of metabolic stress, both of which will enhance the maturation of oocytes (25).

Consequently, the faster oocyte maturation rates of Awassi animals are related to the interaction of genetic, hormonal, cellular, and environmental characteristics and, therefore, make this breed a likely candidate for reproductive enhancement programs and artificial insemination (AI) programs.

Figure 4 provides additional evidence of the difference in development of Awassi vs. Hamdani oocytes because of the statistically significantly reduced number of ovulated oocytes from course- and cumulus-denuded oocyte populations of Hamdani females compared to the same cumulus-enclosed population of Awassi females.

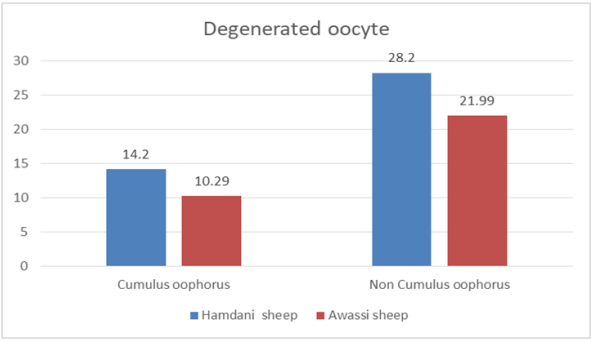

Figure 4. *Percentage of Degenerative Oocytes with/respect to Cumulus Oophorus within Awassi vs. Hamdani Sheep*

The study's observations revealed that female Awassi had statistically significant superiority in having fewer oocytes that were developmentally incompetent due to being associated with cumulus cells (Figure 5) as

compared to those remaining denuded from any cumulus cells. The improvement in this area is attributed to the function of the cumulus oophorus cellular populations that surround the oocyte, which provide important connections to support the oocyte microenvironment and produce the regulatory factors necessary for maturation of the oocyte. These somatic cells are critical in providing nutrient transfer and androgenic signal transduction from the follicular environment to the oocyte, thereby facilitating meiotic progression and cytoplasmic maturation (28). The absence of cumulus cells surrounding an oocyte or impaired cumulus cell activity can disrupt intercellular communication homeostasis between an oocyte and its immediate environment; thus, inhibiting an oocyte's ability to be competent for development, or rendering it susceptible to degeneration (27). Cumulus cell presence indicates the developmental readiness of an oocyte—by extension, cumulus cells are critical for the structural and functional maturation of oocytes. Cumulus cell absence limits the ability of an oocyte to receive sufficient metabolic support (e.g., energy substrate, ATP production) or apoptosis (programmed cell death) and antioxidant protection against oxidative stress. The action mechanisms described above have been associated with the increased frequency of degeneration within cumulus cell-free oocyte populations (29).

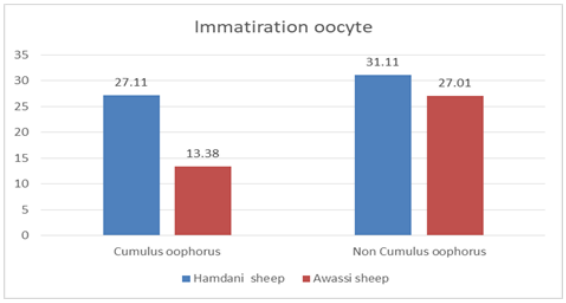

Figure 5. Percentage of immature Oocytes with/respect to Cumulus Oophorus within Awassi vs. Hamdani Sheep

The Relationship Between (LH) and (FSH) Hormone Levels and Oocyte Maturation

In this experimental study, it was shown that high levels of circulating (LH) and (FSH) hormone positively correlate with an increased percentage of developmentally competent oocytes. The data presented in this study indicate that genetic lines that produced the highest concentrations of gonadotropins had significantly more mature oocytes as well as much lower numbers of immature or degenerate oocytes compared to those animals that were lower in circulating LH and/or FSH, such as the Hamdani breed. Further, the Hamdani breed had a lower concentration of (LH) and (FSH) which resulted in having both fewer mature or developmentally competent oocytes and more developmentally incompetent or degenerate oocytes. The results support the promulgation that stimulating the gonads with the proper balance of LH and FSH - specifically moderate elevations of (LH) and (FSH) hormone - is necessary for completing follicular maturation and producing good quality oocytes.

The results from this study support previous findings from Hillier (2001) (30) that FSH is responsible for stimulating follicular growth as well as the production of estrogen, while LH plays a critical role in completing meiosis by triggering ovulation. In support of this, Filicori et al. (2002) (31) showed that there were higher numbers of developmentally competent oocytes during stimulation protocols that utilized sufficient concentrations of LH and therefore were more responsive to developing follicles. Finally, Fauser et al. (2011) (32) also demonstrated a relationship between the levels of (LH) and (FSH) and the maturation of oocytes, and irregular ovulatory patterns in some genetic lines or breeds – such as the Hamdani line.

The positive correlation between (LH) levels and oocyte maturity suggests that (LH) contributes greatly to the improvement of oocyte quality and thus the success of all assisted reproductive technologies as well as that genetically different livestock lines have variable molecular expression of (LH) and (FSH) receptor in the ovary. The results support and corroborate the findings of prior studies concerning polymorphisms in the FSH receptor gene and further demonstrate their impact on reproductive responsiveness (33).

It is apparent from this research and subsequent findings that understanding how the hormones, specifically LH, function in stimulating oocyte development is essential for achieving successful reproductive outcomes. Additionally, it would appear that breed-specific hormonal management protocols are also necessary to maximize the reproductive performance of native ewes. All of these findings will ultimately lead to creating more efficient breeding programs for assisted reproductive technology in agricultural production systems.

Conclusions

The Awassi breed had higher levels of hormone responsiveness than Hamdani animals. The Awassi breeds had greater amounts of circulating (FSH) and (LH) after reproductive stimulation protocols and this positively affected the development and rate of maturation of oocytes. A significant relationship between (FSH) and (LH) and the number of oocytes that were developed was found in both the Awassi and Hamdani breeds indicating that these gonadotropins are essential regulators of oocyte maturation in the last stages. The Awassi breed was the most efficient with respect to hormonal responsiveness and oocyte development, making them an ideal candidate for fertility enhancement programs/assisted reproductive technologies in the Iraqi sheep's production systems.

Recommendations

Given the established greater reproductive efficacy of Awassi over Hamdani animals, implementation of Awassi genotypes in assisted reproductive technology programs and embryo transfer programs in different Iraqi geographic locations should occur. Furthermore, additional research studies addressing other native sheep breeds will be required to assess their comparative hormonal response to seasonal, nutritional, and climatic environmental conditions so that there is a better understanding of fertility characteristics of Iraqi sheep populations. Future research efforts should include additional reproductive hormones, such as estrogen and progesterone, to develop a greater overall understanding of factors affecting oocyte maturation.

In order to enhance our understanding of follicular cellular components and functional interbreed differences at the cellular level, the development of enhanced molecular analyses and gene expression technologies (such as reverse transcription polymerase chain reaction) is recommended to continue investigations of (LH) and (FSH) receptor populations.

Development of breed-specific hormone stimulation protocols is recommended to optimally utilize assisted reproductive programs and increase ovulation rates and conception success rates.

References

1. Fortune, J. E. (2016). Ovarian follicular growth and development in mammals. Biology of Reproduction, 94(4), 86. https://doi.org/10.1095/biolreprod.115.134817
2. Ginther, O. J., Bashir, S. T., Hoffman, M. M., & Beg, M. A. (2018). Hormonal and follicular dynamics during the estrous cycle in sheep. Theriogenology, 112, 1–10. https://doi.org/10.1016/j.theriogenology.2018.01.034
3. Webb, R., & Campbell, B. K. (2007). Development of the dominant follicle: mechanisms of selection and maintenance of oocyte quality. Theriogenology, 68(S1), S9–S15. https://doi.org/10.1016/j.theriogenology.2007.04.036
4. Scaramuzzi, R. J., et al. (2011). A review of the effects of supplementary nutrition in the ewe on the concentrations of reproductive hormones. Reproduction Nutrition Development, 46(4), 339–354. https://doi.org/10.1051/rnd:2006021
5. Monniaux, D., Clément, F., Dalbiès-Tran, R., Estienne, A., Fabre, S., Mansanet, C., & Rico, C. (2014). The ovarian reserve of primordial follicles and the dynamic reserve of antral growing follicles: what is the link? Biology of Reproduction, 90(4), 85. https://doi.org/10.1095/biolreprod.113.117770
6. Baird, D. T. (2018). Endocrinology of follicular development and ovulation. Animal Reproduction, 15(3), 278–291.
7. Mossa, F., Duffy, P., Walsh, S. W., Butler, S. T., Berry, D. P., Carter, F., & Evans, A. C. O. (2012). Differences in follicular dynamics, gonadotropin and steroid concentrations, and progesterone receptor expression in high vs. low fertility cows. Reproduction, 143(5), 625–633. https://doi.org/10.1530/REP-11-0415
8. Al-Fatlawi, A. A., Al-Fatlawi, K. M., & Al-Husseiny, M. I. (2020). A comparative study on the reproductive performance of Awassi and Arabi ewes. Iraqi Journal of Veterinary Sciences, 34(2), 265–270.
9. Al-Khuzai, A. L., Al-Jobory, H. T., & Abbas, S. A. (2021). Reproductive performance of Awassi and Arabi ewes under Iraqi environmental conditions. Iraqi Journal of Veterinary Sciences, 35(2), 263–270.
10. Abecia, J. A., Forcada, F., & González-Bulnes, A. (2012). Hormonal control of reproduction in small ruminants. Animal Reproduction Science, 130(3–4), 173–179. https://doi.org/10.1016/j.anireprosci.2012.01.010
11. Bartlewski, P. M., Beard, A. P., & Rawlings, N. C. (2011). Ovarian function in sheep. Animal Reproduction Science, 126(1-2), 1–14. https://doi.org/10.1016/j.anireprosci.2011.04.006
12. Lonergan, P., & Fair, T. (2014). The ART of studying early embryo development: progress and challenges in ruminant embryo culture. Theriogenology, 81(1), 49–55. https://doi.org/10.1016/j.theriogenology.2013.09.022

13. Paramio, M. T. (2010). In vitro maturation of oocytes in domestic ruminants. Animal Reproduction, 7(3), 162–169.
14. National Research Council. 2007. Nutrient Requirements of Small Ruminants: Sheep, Goats, Cervids, and New World Camelids. Washington, DC: The National Academies Press. 384 p. ISBN: 978-0-309-47324-8. DOI: 10.17226/11654
15. Ginther, O. J., Bashir, S. T., Hoffman, M. M., & Beg, M. A. (2018). Hormonal and follicular dynamics during the estrous cycle in sheep. Theriogenology, 112, 1–10. https://doi.org/10.1016/j.theriogenology.2018.01.034
16. Souza, C. J. H., Campbell, B. K., & Baird, D. T. (2002). Follicular dynamics and ovarian steroid secretion in sheep. Reproduction, 123(6), 917–925.
17. Titi, H. H., Alnimer, M. A., & Obeidat, B. S. (2020). Reproductive responses of ewes to synchronization protocols. Animal Reproduction Science, 216, 106357. https://doi.org/10.1016/j.anireprosci.2020.106357
18. Nogueira, D., Vanhoutte, L., Dumortier, F., & De Sutter, P. (2009). Assessment of a new in vitro maturation system for mouse and human cumulus-enclosed oocytes: Three-dimensional prematuration culture in the presence of a phosphodiesterase 3-inhibitor. Human Reproduction, 24(8), 1946–1959. https://doi.org/10.1093/ humrep/dep104
19. Carvalho, A. A., Faustino, L. R., Castro, S. V., et al. (2013). Tissue thickness may influence the outcome of vitrification of goat ovarian cortex. Acta Scientiae Veterinariae, 41, 1150.
20. Lucci, C. M., et al. (2007). Osmotic tolerance and freezability of isolated caprine early-staged follicles. Reproduction, Fertility and Development.
21. Rodrigues, J. K., et al. (2015). Effect of hormonal supplementation on in vitro maturation of caprine oocytes. Theriogenology, 83(1), 124-130.
22. SAS. (2018). Statistical Analysis System, User's Guide. Statistical. Version 9.6th ed. SAS. Inst. Inc. Cary. N.C. USA.
23. Foster, D. L., & Hileman, S. M. (2015). Puberty in sheep. In Knobil and Neill's Physiology of Reproduction (pp. 1257–1306). Elsevier. https://doi.org/10.1016/B978-0-12-397175-3.00025-2
24. McNatty, K. P., et al. (2002). Ovarian follicular development and function: the role of FSH and LH in regulating the expression of steroidogenic enzymes. Reproduction, 123(5), 743–751. https://doi.org/10.1530/rep.0.1230743
25. Scaramuzzi, R. J., et al. (2006). A review of metabolic and endocrine factors influencing the reproductive system in sheep. Reproduction Nutrition Development, 46(4), 339–354. https://doi.org/10.1051/rnd:2006021
26. Notter, D. R. (2008). Genetic aspects of reproduction in sheep. Reproduction in Domestic Animals, 43(Suppl. 2), 122–128. https://doi.org/10.1111/j.1439-0531.2008.01150.x
27. Gilchrist, R. B., Lane, M., & Thompson, J. G. (2008). Oocyte-secreted factors: regulators of cumulus cell function and oocyte quality. Human Reproduction Update, 14(2), 159–177. https://doi.org/10.1093/humupd/dmm040
28. Eppig, J. J. (1991). Intercommunication between oocytes and companion somatic cells in the ovary. BioEssays, 13(11), 569–574. https://doi.org/10.1002/bies.950131102
29. Sugiura, K., & Eppig, J. J. (2005). Control of metabolic cooperativity between oocytes and their companion granulosa cells. Reproduction, Fertility and Development, 17(7), 667–674. https://doi.org/10.1071/RD05068
30. Hillier, S.G. (2001). Gonadotropic control of ovarian follicular growth and development. Mol Cell Endocrinol, 179(1-2), 39–46. https://doi.org/10.1016/S0303-7207(01)00473-2
31. Filicori, M., et al. (2002). Luteinizing hormone activity in menotropins optimizes folliculogenesis. J Clin Endocrinol Metab, 87(3), 1156–1161. https://doi.org/10.1210/jcem.87.3.8275
32. Fauser, B.C., et al. (2011). Women's health aspects of PCOS: 3rd PCOS Consensus. Fertil Steril, 97(1), 28–38.e25. https://doi.org/10.1016/j.fertnstert.2011.09.024
33. Simoni, M., Gromoll, J., & Nieschlag, E. (2002). The FSH receptor: biochemistry, molecular biology, physiology, and pathophysiology. Endocrine Reviews, 23(6), 747–773. https://doi.org/10.1210/er.2001-0023