BF-1806
[1-6]

RESEARCH ARTICLE Indian Journal of Animal Research

Differential Effects of Reproductive Hormones on the Developmental Potential of Oocytes in Hainan Black Lambs/ Goats

Pei Lin-Lin[1], Wang Ruo-Tong[1], Wang Wen-Hao[1], Hu Kai[1], Xu Xin[1], Liu Chun-Jie[1] 10.18805/IJAR.BF-1806

ABSTRACT

Background: The study elucidated the effects of reproductive hormones on the developmental potential of oocytes in Hainan lamb/ adult goats by analyzing the changes in the concentrations of reproductive hormones (FSH, LH, 17β-E_2) in Hainan lamb/adult goat serum.

Methods: The experiment used radioimmunoassay to detect follicle stimulating hormone (FSH), luteinising hormone (LH), oestradiol (17β-E_2), concentrations in the serum of Hainan goats (3-month-old/18-month-old) during different oestrous cycles and to analyze the changing pattern of hormones.

Result: The results showed that the number of follicles obtained in lambs was significantly higher than in adult goats (P<0.05), but the quality of follicles in lambs was poor and the number of available oocytes in them was lower than in adult goats. During the estrous cycle, adult black goats exhibit peak serum concentrations of FSH and LH on the 13th day, reaching 2.73 ng/mL and 5.69 ng/mL, respectively. In lamb goats, FSH reaches its peak concentration on the 15th day at 2.56 ng/mL, while LH peaks on the 13th day at 5.13 ng/mL. On the 15th day, both adult black goats and lamb goats experience the lowest serum concentrations of 17β-E_2, at 4.42 ng/mL and 7.63 ng/mL, respectively. The serum concentrations of FSH and 17β-E_2 in adult black goats are significantly higher than those in lamb goats (P<0.05) In summary, FSH and 17β-E_2 can promote the maturation and development of oocytes in Hainan 3-month-old lamb goats, which can be injected with the corresponding concentration of hormones on the 15th day during the synchronized estrus in Hainan 3-month-old lamb goats, to improve the quality and quantity of oocytes, to better utilize the in vitro embryonic production performance of the young animals and to accelerate the acquisition of good livestock breeds.

Key words: Estrous cycle, Hainan black goat, Oocytes, Reproductive hormones.

INTRODUCTION

Differences in fertility seriously constrain the development of the goat industry, the current goat fertility is low, to find the causes of differences in fertility, the study of goats in the estrous cycle of the pattern of change of the changes in the various reproductive hormones, has important theoretical and practical significance. Currently, there are fewer studies on the changing patterns of reproductive hormones during the estrous cycle in goats. Young animals in vitro embryo production technology is the integrated application of a variety of technologies, which has great application prospects in livestock breeding (Ferré *et al*., 2020), tapping the reproductive potential of young animals, rapidly obtaining good livestock breeds, expanding the population of good livestock breeds and shortening the generation interval of good livestock breeds. A large number of young animals oocytes can be obtained quickly after supernumerary ovulation of young animals (Sundaram *et al*., 2012), but the disadvantages of this technique should not be ignored, low maturation rate of young animals oocytes and high developmental abnormality rate (Liu *et al*. 2023). Hainan black goat as Hainan Province, unique to the excellent goat breeds, with roughage, high temperature and humidity, strong disease resistance, early sexual maturity, good meat performance and other advantages (Hua *et al*., 2019). The conservation of their germplasm resources is important for the maintenance of biodiversity and sustainable agricultural development. However, long-term inbreeding and the deterioration of the breeding environment have led to the degradation of breed characteristics, which urgently needs to be protected and improved through scientific research.

The physiological activities of animals are influenced by hormonal regulation, with reproductive hormones having a profound effect on their reproductive performance (Xu *et al*., 2022). Studies have shown that FSH promotes follicular

[1]College of Animal Science and Technology, Tarim University, Key Laboratory of Livestock and Forage Resources Utilization around Tarim, Ministry of Agriculture and Rural Affairs, Alaer 843300, China.

Corresponding Author: Xu Xin, Liu Chun-Jie; College of Animal Science and Technology, Tarim University, Key Laboratory of Livestock and Forage Resources Utilization around Tarim, Ministry of Agriculture and Rural Affairs, Alaer 843300, China.
Email: 1241141697@qq.com; guilt369@163.com

How to cite this article: Lin-Lin, P., Ruo-Tong, W., Wen-Hao, W., Kai, H., Xin, X., Chun-Jie, L. (2024). Differential Effects of Reproductive Hormones on the Developmental Potential of Oocytes in Hainan Black Lambs/Goats. Indian Journal of Animal Research. 1-6. doi: 10.18805/IJAR.BF-1806.

Submitted: 25-03-2024 **Accepted:** 10-10-2024 **Online:** 09-12-2024

growth and maturation and affects the meiotic and developmental capacity of oocytes (Morton *et al*., 2023). LH may play a major role in the development of dominance selection of follicles (Lévy *et al*., 2000) and 17β-E_2 plays an important role in the molecular regulation of follicle formation and early development (Chakraborty and Roy, 2017; Yan *et al*., 2024). Researchers have boosted the number of goat oocytes and *in vitro* maturation rate by modulating hormone concentrations (Avelar *et al*., 2012). However, *in vitro* matured oocytes are still significantly inferior to naturally matured oocytes *in vivo* and the quality of the two is not comparable, highlighting the challenges of current in vitro maturation techniques. For a long time, the low maturity rate and insufficient development potential of lamb oocytes have been the key factors restricting the improvement of their utilization efficiency. In order to investigate this problem, we carried out a study on lamb oocytes and comparatively analyzed the differences in development potential between lamb oocytes and adult oocytes, with a view to providing a scientific basis and an effective strategy for the improvement of the utilization efficiency of lamb oocytes. In this study, we obtained a large number of oocytes through induced supernumerary ovulation, analyzed the differences in maturation and developmental potential between lamb and adult goat oocytes and monitored changes in reproductive hormone concentrations (FSH, LH and 17β-E_2) in both lambs and adult goats during the oestrous cycle, to provide a reliable basis for understanding the mechanisms by which FSH, LH and 17β-E_2 influence follicular development in lambs. This has significant economic implications for improving oocyte utilization rates and provides a foundation for the practical application of supernumerary ovulation technology, aiding in the rapid expansion of the goat population.

MATERIALS AND METHODS

Test animals

The test animals were Hainan black goats and the ewes selected were adult ewes (18 months old) and lambs (3 months old) with normal reproductive function and similar body condition. The ewes were divided into two groups of 20 ewes each, one group of adult ewes with an average weight of (60.5±2.5) kg and the other group of lamb ewes with an average weight of (8±3) kg. All test animals were subjected to the same husbandry management conditions throughout the assay period to ensure the scientific validity and reliability of the test. Three lambs from each of the three weight groups (5-6 kg, 7-8 kg, 9-10 kg) were additionally selected for subsequent trials.

Pregnant horse serum gonadotropin (PMSG) for injection and FSH for injection were from Ningbo Hormone Products Co Ltd, LH kit, FSH kit and 17β-E_2 kit were from Tianjin Jiuding Medical Bio-engineering Co Ltd and progesterone vaginal suppositories (CIDR), were purchased from CHH Plastic Moulting Co, New Zealand.

Estrus detection

Estrus was identified using the estrus test method, observation method and microscopic examination of sections. 12-48 h after PMSG injection, using rams with wrapped penises for estrus teasing, perform 3~4 times daily to observe the estrus condition of the ewes. Take the ewe accepting the ram climbing across as the criterion of estrus and at the same time observe whether the vulva of the ewe is red and swollen, with or without mucus outflow and apply slice microscopy to observe the goat germ cells to assist in judging the stage of estrus.

Supernumerary ovulation

As shown in Fig 1, On Day 1, perform the insertion of vaginal sponge. On Day 9, remove the vaginal sponge from adult/lamb goats. Begin the injection of FSH (40 IU/ml) on Day 15, for four consecutive days, with two injections per day and administer PMSG intramuscularly during the 7th FSH injection. Day 19 estrus teasing followed by insemination for all adult/lamb goats. After superovulation, extract oocytes from each follicle by aspiration.

Collection of blood samples and serum preparation

To determine FSH, LH and 17β-E_2 concentrations in goats serum, we drew 5 mL of blood samples from their jugular veins and preserved them in lithium heparin tubes. Blood serum was separated using a centrifuge at 4000 rpm/10 min and stored at -20°C for later use.

Measurement of serum levels of reproductive hormones

The concentrations of serum FSH, LH and 17β-E_2 were determined by radioimmunoassay (RIA). Among them, the detection limit of LH was 0.01ng/mL, with an intra-batch coefficient of variation of 13.6%; the detection limit of FSH was 0.1ng/mL, with a coefficient of variation of 12.2%; and the detection limit of 17β-E_2 was 0.39 pg/mL, with an intra-batch coefficient of variation of 20.5%. All the test steps should be performed in strict accordance with the instructions of the kit.

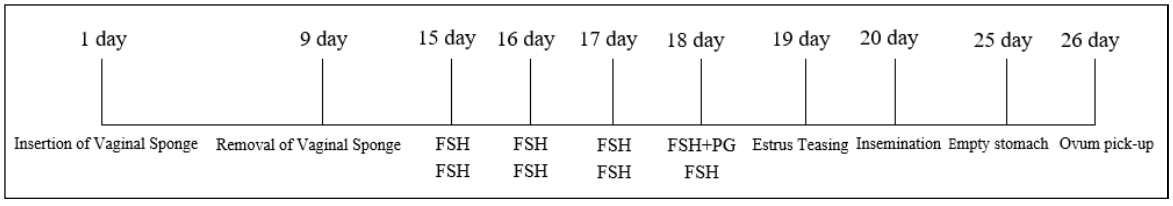


Fig 1: Schematic diagram of superovulation.

Statistical analysis of data

SPSS 21.0 was used to calculate the data and all data were expressed as Mean±SEM. T-tests were performed on the data results to assess the correlation analysis, followed by Duncan's test. $P<0.01$ is considered highly significant, $P<0.05$ is considered significant and $P>0.05$ is not significant.

RESULTS AND DISCUSSION

Hormone expression during the estrous cycle in goats

Hormone expression during the estrous cycle in goats is shown in Fig 2.

Effect of different weights of lambs on supernumerary ovulation

In this experiment, three lambs of each of the three body weight groups (5-6 kg, 7-8 kg and 9-10 kg) were selected and given equal doses of FSH to observe the effect of supernumerary ovulation. The results, as shown in Table 1, did not produce a significant difference ($P>0.05$) in the effect of FSH on super-excretion in these different body weight groups of lambs, despite differences in lamb weight.

Effect of FSH+PMSG injection on the effect of superdischarge

Further studies compared the results of superovulation in adult and lamb goats after FSH+PMSG injections (Fig 3). The results showed that the number of follicles was significantly higher in lamb goats than in adult goats ($P<0.05$), but remarkably, the number of available oocytes was lower in lamb goats than in adult goats (Table 2).

Differential analysis of serum levels of FSH, LH and 17β-E_2 in adult goats and lambs

The results of the experiment are shown in Fig 4, Fig A shows that the FSH concentration on day 1 was significantly lower in adult goats than in lambs ($P<0.05$). At day 13, adult goats FSH reached a maximum concentration of 2.73 ng/mL. And lambs FSH reached a maximum concentration of 2.56 ng/mL on day 15. Fig B shows that both adult goats and lambs had the lowest LH concentrations on day 1. The difference between the two LH concentrations was highly significant ($P<0.01$) at day 7, when lambs reached a maximum LH concentration of 6.83 ng/mL. On day 13, adult goats LH reached a maximum concentration of 5.69 ng/mL. Fig.C shows that 17β-E_2 concentrations were significantly higher in adult goats than in lambs ($P<0.05$) during the oestrous cycle. Adult goats and lambs 17β-E_2 both showed the lowest concentrations of 4.42 ng/mL and 7.63 ng/mL on day 15.

Body weight, as one of the measures of animal growth and development (Liang *et al.,* 2018), may influence the sensitivity of lambs to reproductive hormones. In order to more accurately reveal the specific effects of FSH on lambs of different weights and to gain a deeper understanding of the optimal weight range for lambs in order to optimize hormone utilization efficiency, it is necessary for us to conduct in-depth research. This exploration aims to provide scientific basis for achieving more precise animal husbandry management and improving lamb reproductive performance by analyzing the effects of superovulation in lambs of different weights. The results of this experiment showed that the difference in the number of follicles obtained from superovulation of lambs with different body weights was not significant ($P>0.05$) and that body weight did not have a significant effect on oogenesis in lambs. Therefore, when selecting lambs for superovulation in production, it is possible to select lambs with different body weights to further validate that the main factor affecting ovarian and follicular development is hormones and not body weight.

Monthly age is an important factor influencing ovarian development and follicle numbers in goats. Lambs have great individual variation in their response to exogenous hormone stimulation, with collected oocytes being less developmentally competent than adult ewes (Liu *et al.*, 2023). FSH treatment of lambs stimulates the growth of large numbers of follicles, but they are prone to underdevelopment during subsequent in vitro development (Tian *et al.*, 2022). It was shown that the age of the lambs as well as the FSH

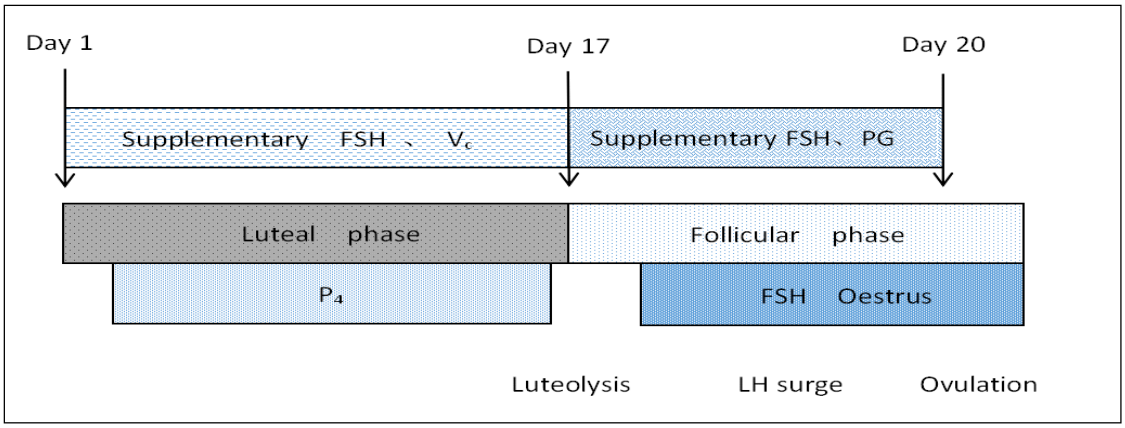


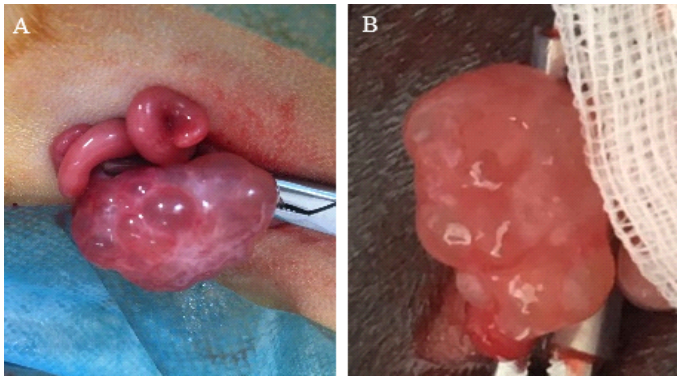
Fig 2: Hormonal expression chart during the estrous cycle of goats.

dose had a significant effect on the number and quality of follicles collected (Valasi *et al*., 2007). During supernumerary ovulation, exogenous FSH effectively promotes the development of a large number of follicles to the ovulatory stage (Vegetti and Alagna 2006). The number of potentially ovulatory follicles in the ovary far exceeds the number of ovulations induced by endogenous FSH and the supplementation of exogenous PMSG can enhance this process, promoting the maturation and ovulation of all follicles with ovulation potential, thereby significantly improving ovulation rate. The main purpose of supernumerary ovulation in lambs is to maximise oocyte acquisition. Although the number of follicles after supernumerary ovulation in lambs was significantly higher than that in adult goats ($P<0.05$), the difference in the number of available oocytes was not significant compared with that in adult goats ($P>0.05$), suggesting that lambs have many follicles but their quality varies. This phenomenon may be attributed to the high sensitivity of lambs to exogenous FSH (Tassell *et al*. 1978). In addition, it was noted that the rate of meiosis at oocyte maturity in young animals was similar to that of adult animals and that there were no significant differences in morphology and total cell number of blastocysts generated from in vitro cultures (Kochhar *et al*., 2010), illustrating the potential for oocyte production in lambs.

The follicle selection process is mainly regulated by FSH (Liu *et al*., 2017). When a cavity is formed inside the follicle, FSH not only promotes the continued development and enlargement of the follicle, but also accelerates its maturation process. FSH, as a key hormone secreted by the pituitary gland, plays a crucial role in regulating growth and development, sexual maturation and maintenance of reproductive system homeostasis in goats under normal physiological conditions. However, abnormally elevated FSH levels may instead be detrimental to ovarian function and lead to ovarian hypoplasia (Hussein *et al*., 2017). LH is secreted by adenopituitary eosinophils and interacts with FSH to influence follicular growth and development, maturation and the process of ovulation. The selection phase of dominant follicles is often accompanied by a decrease in FSH concentration, with ovulation or follicular atresia occurring when FSH reaches its lowest concentration. Notably, there appears to be a transition point from FSH dominance to LH dependence at the final stage of dominant follicle selection, but the exact

Table 1: Number of follicles obtained from superovulation in lambs of different body weights.

Weight	5-6 kg	7-8 kg	7-8 kg
Follicle Count	69 ± 1.1^{ns}	77 ± 1.7^{ns}	72 ± 1.4^{ns}



Note: Fig A represents the superovulated follicles in the right ovary of adult goats and Fig B represents the superovulated follicles in the right ovary of lamb goats.

Fig 3: Superovulated follicles morphology in adult goats/lamb goats.

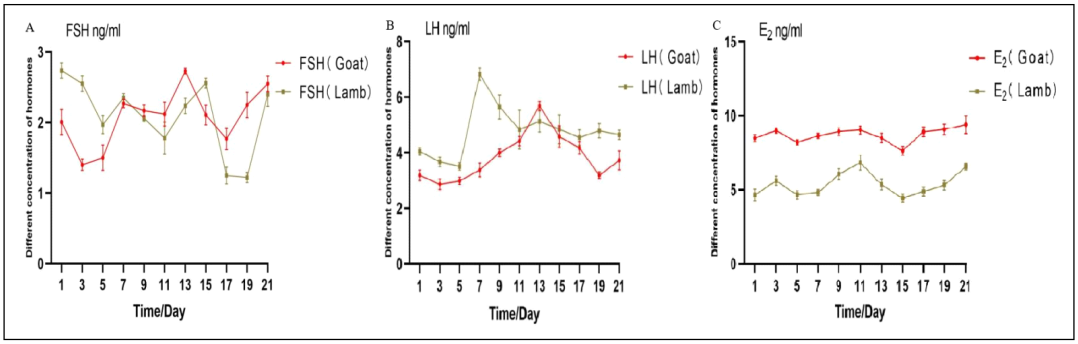


Fig 4: Fluctuations in concentrations of FSH, LH and 17β-E_2 during the estrous cycle.

Table 2: Superovulation results statistics for adult goats/lamb goats.

Category	Adult goats	Lamb goats
Number of goats	20	20
Treatment method	FSH (4x40mg) + PMSG (250IU)	FSH (4x40mg) + PMSG (250IU)
Follicle count (3-8 mm)	80.6±16.2[a]	102.1±7.2[b]
Oocyte count	1480	1445

Note: Different superscript letters (a, b) indicate significant differences between groups.*

mechanism and timeframe of this is not entirely clear. The role of LH is particularly critical, as it induces further growth of dominant follicles and secretion of 17β-E_2, mainly by regulating the frequency of LH pulses. The prolongation of the LH pulse may contribute to keeping the dominant follicle active for a longer period of time (Diskin *et al*., 2003). In the present study, we found that the serum levels of FSH and LH peaked on the day of Removal of Vaginal Sponge in adult Hainan black goats, whereas in lambs, the peaks occurred two days after Removal of Vaginal Sponge, suggesting that these two time points may be the critical nodes for follicular wave formation in adult goats and lambs, respectively. In addition, the present study found that the mean secretion level of LH was highly significantly higher ($P<0.01$) than that of FSH during the oestrous cycle in both adult goats and lambs, a finding that is consistent with previous findings on FSH and LH (Fleming *et al*., 1995), further emphasising the central role of LH in follicular development and ovulation.

Estrogens mainly include 17β-E_2, estrone (E1) and estriol (E3), (Wang *et al*. 2024), with 17β-E_2 exhibiting the most significant biological activity (Carrillo *et al*. 2017). 17β-E_2 has a short half-life in the body, between 5 and 20 minutes and is mainly secreted by tissues such as the follicle, corpus luteum and placenta and is a core oestrogen that regulates the development of the body's sex organs and secondary sex characteristics (Kaczynski *et al*. 2021). It has been shown that the concentration of 17β-E_2 is strongly correlated with follicle size and increases accordingly with increasing follicle volume (Lawrenz *et al*., 2020). 17β-E_2 levels were significantly higher in dominant follicles at later stages of development than at earlier stages. During the oestrous cycle, 17β-E_2 levels were significantly higher in adult goats than in lambs ($P<0.05$) and the level of 17β-E_2 content directly determined the occurrence of follicular atresia. Specifically, high levels of 17β-E_2 are effective in stimulating the maturation and development of multiple follicles in the ovary, thereby boosting the number of effective oocytes and consequently the reproductive rate. In adult goats and lambs 17β-E_2 showed a minimum within two days after Removal of Vaginal Sponge, a process that regulates FSH and LH secretion through a negative feedback mechanism, resulting in peaks of FSH and LH, which are essential for promoting ovulatory development in goats. Subsequently, the concentration of 17β-E_2 rapidly rebounded and peaked, which signalled that Hainan black sheep entered the active period of oestrus and ovulation. Therefore, in order to improve reproductive efficiency, the optimal time for artificial insemination should be selected to start the day after Removal of Vaginal Sponge to fully utilise the follicles.

CONCLUSION

In the present study, an in-depth study was conducted on Hainan black goats, revealing that lambs possess a high number of follicles, albeit of poor quality. In contrast, adult goats exhibit fewer follicles, but these are of superior quality. The maturation and development of oocytes in lambs can be significantly enhanced through the combined administration of FSH and 17β-E_2. It is recommended that reproductive hormone assays be performed and the concentrations of injections be adjusted on day 15 to optimize both the quantity and quality of oocytes. Removal of Vaginal Sponge Within 48 hours is the peak period of estrus and ovulation of Hainan black goats. Artificial insemination during this period has the best effect.

ACKNOWLEDGEMENT

This study was funded by the Tarim University President's Fund Mechanism of Hormone-Mediated Glucose Metabolism in Cele Black Sheep Cavity Follicles *in vitro* and loyal (TDZKBS202102), precision editing in the sheep genome using the PE system (TDZKBS202417), study of transcriptome methylation on selection for reproductive performance in sheep and application of timed insemination technology (2023A02011), thanks are due to Tarim University for providing the experimental equipment.

Declaration of completing interest

The authors do not have competing interests that interfere with the independence and rigor of these studies.

REFERENCES

Avelar, R.Q., Teixeira, D.I.A., Freitas, V.J.F., Oliveira, J.T.A., Melo, L.M. and Pereira, A.F. (2012) Oocyte production and *in vitro* maturation in Canindé goats following hormonal ovarian stimulation. Animal Reproduction. 9(1): 27-32.

Chakraborty, P., Roy, SK. (2017). Stimulation of primordial follicle assembly by estradiol-17β requires the action of bone morphogenetic protein-2 (BMP2). Sci Rep. 7(1): 15581. Published 2017 Nov 14.

Carrillo, E., Meza, Herrera, C.A., Luna, Orozco, J.R., Delgado, Gonzales, R.A, Gaytan. Aleman, L.R., Garcia, O.A,, Veliz, F.G. (2017), Contreras Villarreal V. Evaluation of out-of-season estrus induction protocols in progesterone-primed mix-breed dairy goats using eCG, GnRH and E2. Indian Journal of Animal Research. 53: 711-715.

Diskin, M.G., Mackey, D.R., Roche, J.F., *et al*. (2003). Effects of nutrition and metabolic status on circulating hormones and ovarian follicle development in cattle [J]. 78(3-4): 345-370.

Ferré, L.B., Kjelland, M,E., Strøbech. L.B., Hyttel, P., Mermillod, P., Ross, P.J. (2020). Review: Recent advances in bovine *in vitro* embryo production: Reproductive biotechnology history and methods. Animal. 14(5): 991-1004.

Fleming, J.S., Greenwood, P.J., Heath, D.A. (1995) *et al.* Expression of gonadotrophin subunit genes in sheep that were homozygous carriers and non-carriers of the Booroola fecundity gene FecB [J]. J Reprod Fertil. 103(2): 315-21.

Hua, R., Zhou, L., Zhang, H., Yang, H., Peng, W., Wu K. (2019). Studying the variations in differently expressed serum proteins of hainan black goat during the breeding cycle using isobaric tags for relative and absolute quantitation (iTRAQ) technology. J. Reprod Dev. 65(5): 413-421.

Hussein, A.M., Al-Shakaili, Y.O., Al-Ismaily, A.N., Al-Alawi, H.H. (2017). Effect of different doses of FSH on superovulation, production and quality of embryo in North Omani Cattle breed. Indian Journal of Animal Research. 51(1): 8-14.

Kochhar, H., W.U.B., Morris, L. *et al.* (2010). Maturation Status, Protein Synthesis and Developmental Competence of Oocytes Derived from Lambs and Ewes [J]. Reproduction in Domestic Animals. 37(1): 19-25.

Kaczynski, P., Baryla, M., Goryszewska, E., Waclawik, A. (2021). Estradiol-17β Regulates Expression of Luteal DNA Methyltransferases and Genes Involved in the Porcine Corpus Luteum Function *in vivo*. Int. J. Mol. Sci. 22: 3655.

Liu, K., Zhang, L., Qi Q, Li. J., Yan, F., Hou, J. (2023). Growth hormone treatment improves the development of follicles and oocytes in prepubertal lambs. J Ovarian Res. 2023;16(1): 132. Published.

Liang, Y., Zhu, B., Jin, S., Bao, J., Xu, L., Chen, Y., Gao, X., Zhang, L., Gao, H., Li, J. (2018). The growth curve fitting and the correlation analysis between Body Weight and Body Measurements in Chinese Simmental Beef Cattle Population. Acta Vet. Zootech. Sin. 49: 497-506.

Lawrenz, B., Bixio, L.D., Coughlan, C., *et al*. (2020). Inhibin A-A Promising Predictive Parameter for Determination of Final Oocyte Maturation in Ovarian Stimulation for IVF/ICSI [J]. 11: 307.

Liu, K., Zhang, L., Qi Q, Li, J., Yan, F., Hou, J. (2023). Growth hormone treatment improves the development of follicles and oocytes in prepubertal lambs. J. Ovarian Res. 16(1): 132. Published 2023 Jul 5.

Lévy, D.P, Navarro, J.M., Schattman, G.L., Davis, O.K., Rosenwaks, Z. (2000). The role of L.H. in ovarian stimulation: exogenous LH: let's design the future [published correction appears in Hum Reprod 2001 Mar;16(3):598]. Hum Reprod. 15(11): 2258-2265.

Liu, W., Xin, Q., Wang, X. *et al.* (2017) Estrogen receptors in granulosa cells govern meiotic resumption of pre-ovulatory oocytes in mammals [J]. Cell Death Dis. 8(3): 2662.

Morton, A.J., Candelaria, J.I., McDonnell, S.P., Zgodzay, D.P, Denicol, A.C. (2023). Review: Roles of follicle-stimulating hormone in preantral folliculogenesis of domestic animals: what can we learn from model species and where do we go from here?. Animal. 17 Suppl 1: 100743.

Sundaram, M. Muthuramalingam, T., Rajkumar, J.S.I., Nishanth, B., Sivakumar, T. (2012). Synchronization of ovulation and fixed time breeding in tellicherry goats. Indian Journal of Animal Research. 51(3): 9-11.

Tian, H., Ren, P., Liu, K.(2022) *et al.* Transcriptomic comparison of ovarian granulosa cells between adult sheep and prepubertal lambs. BMC Genomics. 23(1): 151. Published 2022 Feb 21.

Tassell, R., Chamley, W.A., Kennedy, J.P. (1978). Gonadotrophin levels and ovarian development in the neonatal ewe lamb [J]. Aust J Biol Sci. 31(3): 267-273.

Valasi, I., Leontides, L., Papanikolaou, T., Amiridis, GS. (2007). Age, FSH dose and follicular aspiration frequency affect oocyte yield from juvenile donor lambs. Reprod Domest Anim. 42(3): 230-237.

Vegetti, W., Alagna, F., F.S.H. and folliculogenesis: (2006) from physiology to ovarian stimulation. Reprod Biomed Online. 12(6): 684-694.

Wang, Y., Lv, J., Xie, Z., Huai, N., Zhang, K., Zhou, Y., Reze, Y., Zhu, H., Li, X. and Zhang, Z. (2024). Gene expression profiles of Chinese medaka (Oryzias sinensis) primary hepatocytes in response to estrone (E1), 17β-estradiol (E2) and estriol (E3). Journal of Environmental Sciences. 94: 1-10.

Xu, Q., Wang, C., Wang, L. *et al.* (2022). Correlation analysis of serum reproductive hormones and metabolites during multiple ovulation in sheep. BMC Vet Res 18, 290.

Yan, Y., Zhang, H., Xu, R. (2024). *et al.* Single-cell sequencing reveals the transcriptional alternations of 17β-estradiol suppressing primordial follicle formation in neonatal mouse ovaries. Cell Prolif. Published online July 10.