RESEARCH ARTICLE Indian Journal of Animal Research, Volume 58 Issue 5: 791-796 (May 2024)

Morphological Characteristics of Ovarian Tissues and Follicular Fluid Metabolites of Female Lambs and Ewes in Subtropics

A.A. Mohammed[1], S. Al-Suwaiegh[1], I. Al-Gherair[1], S. Al-Khamis[1], S. Al-Awaid[1], H. Al-Sornokh[1], W.F. Alhujaili[2], A. Mohammed[3], A. Mohammed[3]

10.18805/IJAR.BF-1756

ABSTRACT

Background: Oocyte and follicular fluid recovery and evaluation are a crucial step in assisted reproductive technologies. The aims of the current study were to evaluate the ovarian tissues and their resulting oocytes and follicular fluid of Awassi female lambs and ewes in subtropics of Saudi Arabia.

Methods: Thirty reproductive systems of female lambs in addition to forty reproductive systems of ewes of follicular and luteal phases were collected. The ovarian tissues were evaluated through ovarian follicle numbers, oocytes recovery in addition to determination of follicular fluid (FF) metabolites. The ovarian follicles were categorized into small (<3 mm), medium (3-5 mm) and large follicles (>5 mm). The medium and large ovarian follicles were aspirated for FF collection and oocytes recovery. The FF concentrations of total protein (g/dl), urea (mg/dl), glucose (mg/dl), total cholesterol (mg/dl) and triglycerides (mg/dl) were determined.

Result: The weight of reproductive system and ovary were increased of ewes compared to female lambs. The number of small follicles was not differed among groups whereas the numbers of medium and large follicles increase in ewes groups. The highest number of medium follicles was found in ewes of follicular phase compared to other groups. Although the percentage of oocyte recovery increased in ewes compared to female lambs, the oocytes quality was not differed among groups. The total protein values were high ($P<0.05$) in FF of ewes compared to that of female lambs. On the other, the values of urea and triglycerides were low ($P<0.05$) in FF of ewes compared to FF of female lambs. Moreover, the values of total protein, urea and triglycerides were comparable in FF of ewes during follicular or luteal phase. Additionally, there is slight increase of glucose and slight decrease of total cholesterol values in FF of ewes compared to FF of female lambs. In conclusion, follicles of ovarian tissues, oocyte recovery and quality and follicular fluid biochemistry of female lambs and ewes showed differences. The recovered oocytes and FF are available for assisted reproductive techniques.

Key words: Follicular fluid, Follicular phase, Luteal phase, Metabolites, Oocytes, Ovarian follicles.

INTRODUCTION

Small ruminants, including sheep and goats, comprise over 90% of the total livestock population in the Kingdom of Saudi Arabia. According to estimates from the Ministry of Environment, Water and Agriculture in 2021, there were approximately 17.5 million sheep and 6.1 million goats in KSA. In general, the central and northern regions of Saudi Arabia, including the regions around Riyadh and Hail, are known for their significant livestock populations. Additionally, regions with agricultural activity, such as the Al-Qassim and Eastern provinces, also have notable livestock populations (FAO, 2021). The population of small ruminants is not covered the requirements of KSA from milk and protein (GAS, 2021).

The ovaries are organs in the females produced follicles during the reproductive cycles in ruminants (Cerri *et al.*, 2009; Senosy *et al.,* 2017). The ovary contains several hundred of growing follicles (Gordon, 2003). Visible vesicular follicles at different stages of oestrous cycle was recorded and the number of follicles did not differ between the right and left ovaries (Kaulfuss *et al.*, 1994). Sheep are produced one or more oocytes per oestous cycle (Walker *et al.*, 2023). The first oestrus cycle is at the age of 7-9 months in sheep with milk teeth.

[1]Department of Animal and Fish Production, College of Agriculture and Food Sciences, King Faisal University, P.O. Box 402, Al-Ahsa 31982, Kingdom of Saudi Arabia.
[2]Department of Biology, College of Science, Taibah University, Al Madinah Al Munawwarah, 41321, Kingdom of Saudi Arabia.
[3]College of Human Medicine, Assiut University, Egypt.

Corresponding Author: A.A. Mohammed, Department of Animal and Fish Production, College of Agriculture and Food Sciences, King Faisal University, P.O. Box 402, Al-Ahsa 31982, Kingdom of Saudi Arabia. Email: aamohammed@kfu.edu.sa

How to cite this article: Mohammed, A.A., Al-Suwaiegh, S., AlGherair, I., Al-Khamis, S., Al-Awaid, S., Al-Sornokh, H., Alhujaili, W.F., Mohammed, A. and Mohammed, A. (2024). Morphological Characteristics of Ovarian Tissues and Follicular Fluid Metabolites of Female Lambs and Ewes in Subtropics. Indian Journal of Animal Research. 58(5): 791-796. doi: 10.18805/IJAR.BF-1756.

Submitted: 12-01-2024 **Accepted:** 02-03-2024 **Online:** 09-03-2024

Cumulus-enclosed germinal vesicle oocytes can be aspirated from ovarian follicles (Gordon, 2003; Mohammed *et al.*, 2005). They can be used for *in vitro* embryo production through oocyte maturation, fertilization and culture (Stamperna *et al.,* 2020; Ryu *et al.,* 2023). Furthermore, the

follicular fluid aspirated along with oocytes can be used for oocyte maturation and affect positively further embryo development (Mohammed *et al.,* 2005; Contreras-Solís *et al.,* 2021). Oocytes and follicular fluid (FF) availability are essential for development of assisted reproductive techniques (ARTs), which supports reproductive medicine outcomes (Fiscus *et al.,* 2023; Niribili *et al.,* 2023). Therefore, the biochemistry of aspirated follicular fluid was explored (Mohammed 2011; Mohammed *et al*., 2011, 2012, 2019a,b). Therefore, the aims of the current study were to explore the reproductive status of slaughtered female lambs and ewes for collection and evaluation of oocytes quality and follicular fluid biochemistry.

MATERIALS AND METHODS

Site of study and management

The current study was carried out in Al-Ahsaa according to procedure approved by the Ethics Committee of Animal Experimentation of King Faisal University, Saudi Arabi from June to December 2023.

Reproductive system collection and evaluation

The slaughtered animals were categorized according their teeth into lambs (milk teeth) and ewes (2-8 permanent incisors). The collected reproductive systems of the slaughtered animals were transported to the laboratory within 1-2 hours at 30-33°C in thermos. The reproductive systems of adult animals were distributed according to the oestrous cycle stage into follicular or luteal phase. Weights (g) of reproductive system and its ovaries were recorded per animal. The numbers of visible antral follicles/animal were counted in addition to aspiration of medium and large follicles using 18-gauge needle and a syringe. The oocytes were collected under stereomicroscope and were classified into grade (I) oocytes completely surrounded with cumulus-cell layers, grade (II) oocytes surrounded scantly with cumulus-cell layers and grade (III) denuded oocytes (Ganguli *et al*., 1998).

Follicular fluid collection and analysis

Aspirated follicular fluid samples were centrifuged for 15 min. at 2000 r.p.m. Follicular fluid samples were stored at -20°C until further analysis. Follicular fluid samples were analyzed for total protein, urea nitrogen using commercial kits (Diamond Diagnostic). Follicular fluid concentrations of glucose, total cholesterol and triglycerides were determined spectrophotometrically using commercial kits (Vitro Scient; Biochemica und Diagnostica mbH, Germany). The assay procedures were done according to the manufacturer's instructions.

Statistical analysis

The data was presented as mean±SEM according to general linear model (GLM) of SAS program (2008). Differences between lambs and ewes were evaluated in reproductive system characters, follicular fluid biochemistry by one-way ANOVA. Duncan's multiple range tests was used to compare the effect of groups. Level of significance was set at $P<0.05$. Statistical model as follow:

$$Yij = \mu + Ti + Eij$$

Where:
Yij = Experimental observation ij.
μ = Overall mean.
Ti = Effect due to groups i.
Eij = Experimental error.

RESULTS AND DISCUSSION

Characters of reproductive system, ovarian follicle and the resulting oocytes

Weights of reproductive systems and ovaries were increased in ewes compared to lambs. Weights (g) of ovaries were higher in the luteal phase ($P<0.05$) than the follicular phase of ewes. The numbers of small follicles were higher ($P<0.05$) in lambs compared to ewes. Furthermore, the numbers of medium follicles were higher ($P<0.05$) in pubertal sheep during follicular phase compared to other groups. Besides, the numbers of large follicles were higher ($P<0.05$) in ewes compared to female lambs. The quality of oocytes was better of the luteal phase compared with the follicular phase (Table 1). The percentage (%) of recovered oocytes of aspirated follicles was higher in ewes compared to lambs. Oocytes of grade I and II were comparable among groups whereas oocytes of grade III were higher in pubertal sheep (Fig 1).

Follicular fluid biochemistry

The concentrations of total protein (g/dl), urea (mg/dl), glucose (mg/dl), total cholesterol (mg/dl) and triglycerides (mg/dl) are presented in Table (2). The total protein values were high ($P<0.05$) in FF of ewes compared to that of female lambs versus urea values. The values of glucose were high ($P>0.05$) in FF of ewes compared to FF of female lambs. Additionally, the values of total cholesterol and triglycerides were low in FF of ewes compared to FF of female lambs. Finally, the values of determined metabolites were comparable in FF of follicular or luteal phase FF of ewes.

The results of the present study demonstrate the weight of reproductive system and ovary, ovarian follicle numbers in relation to sizes, oocyte recovery, oocyte quality, in addition to follicular fluid biochemistry (Fig 1 and Table 1-2). The weights (g) of reproductive system and ovary were increased in ewes compared to female lambs due to simultaneous increase of body weight and presence of corpora lutea (Mohammed *et al.,* 2012; Mohammed and Kassab 2015). Corpora lutea weight on days 14 was 4.7 g, which represents about 30.1% of the ovarian weight (Fields and Fields, 1996; Osman and Shehata, 2005).

The number of small follicles was not differed among groups whereas the number of medium and large follicles were increased in ewes groups, which might be due to the changes of FSH and LH hormonal values (Gordon 2003; Morton *et al.,* 2023). The highest number of medium follicles was found in follicular phase of ewes group compared to

luteal phase or female lamb groups. This might be attributed to follicular wave emergence, which is primarily controlled by follicle-stimulating hormone during estrous cycle (Bartlewski *et al.,* 2011). In addition, the number of antral follicles is highly variable among animals (Murasawa *et al.,* 2005). The number of antral follicles in heifers and beef cows is influenced by birth weight and age but not by stage of the estrous cycle (Cushman *et al.*, 2009). Although the percentage of oocyte recovery increased in ewes compared to female lambs, the oocytes quality was not differed among groups (Table 1). The higher follicle sizes in ewes than female lambs, the higher pressure inside the follicle resulting in improvement of oocyte recovery and quality (Sarwar *et al.,* 2020).

Follicular fluid plays pivotal roles in follicle growth and development, maturation of oocytes and development of preimplantation embryos (Lopes *et al.,* 2019; Azari-Dolatabad *et al.,* 2021; Gabryś *et al.*, 2022). The values of FF metabolites of female lambs and ewes (total protein, urea, glucose, total cholesterol and triglycerides) are presented in Table (2). Follicular fluid is liquid surrounding the developing oocyte within the ovary. It is originated from blood plasma components and the secretory activity of the oocyte and the surrounding cells (Mohammed *et al.,* 2005; Hashemitabar *et al.,* 2014; Aljubran *et al.,* 2023). Certain biochemical constituents of follicular fluid changes with the follicle growth and maturation to influence various processes (Deka *et al.,* 2014).

The total protein values were high (P<0.05) in FF of ewes compared to FF of lambs due to the high follicle size or follicular wave (Table 1) (Aller *et al.,* 2013). The values of urea in FF were significantly low of ewes compared to female lambs. This finding is in agreement of other studies (Mohammed *et al.,* 2019a,b). This might be attributed to differences in follicle sizes among groups (Table 1). Studies of Leroy *et al.* (2004) and Mohammed *et al.* (2011) indicated that urea concentration decreased from small to large follicles in dairy cows and sheep, respectively.

The values of glucose were slightly high (P>0.05) in FF of ewes compared to FF of female lambs, which might attributed to larger follicle diameter in ewes compared to female lambs (Tabatabaei and Mamoei, 2010; Tabatabaei *et al.,* 2010). Glucose plays a pivotal role in ovarian tissue metabolism because it is the major energy source for the ovarian tissues. There is a possibility that glucose metabolism is less intensive in the large follicles compared

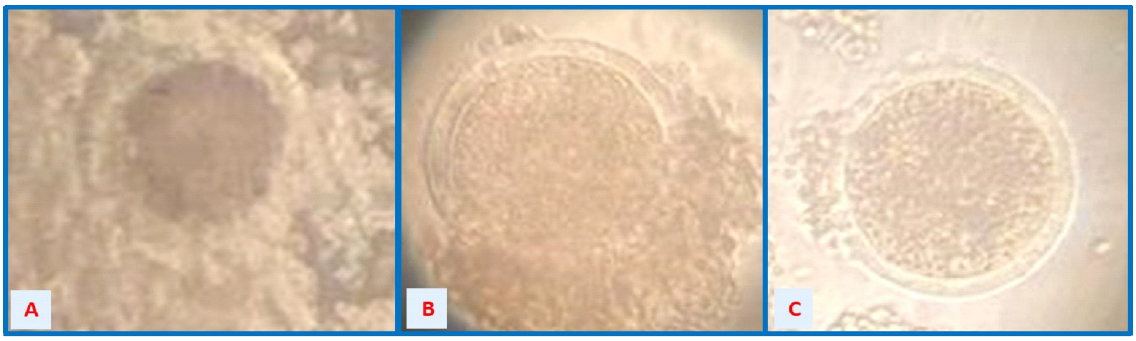

Fig 1: Grades of aspirated oocytes; (A) cumulus-enclosed grade I oocyte, (B) partially cumulus-enclosed grade II oocyte, (C) denuded grade III oocyte.

Table 1: Reproductive systems, ovarian follicles and the resulting grade oocytes of lambs and ewes.

Item	Female lambs	Ewes	
Stage of oestrous cycle	Follicular phase	Follicular phase	Luteal phase
No. of reproductive systems	30	20	20
Reproductive system weight, g	13.80^{b}±2.21	24.18^{a}±2.35	23.85^{a}±2.24
Ovaries weight	0.46^{c}±00.12	0.62^{b}±0.11	1.35^{a}±0.31
No. of follicles	10.50±1.37	12.5±1.83	10.30±1.29
Small follicles, < 3 mm	6.30±0.87	6.4±1.83	5.60±1.24
Medium follicles, 3-5 mm	3.00^{b}±0.55	3.8^{a}±0.83	2.80^{b}±0.65
Large follicles, > 5 mm	1.20^{b}±0.55	2.3^{a}±0.63	1.90^{a}±0.53
No. of aspirated follicles	126	122	94
No. of recovered oocytes, %	57 (45.2%)	72 (59.0%)	54 (57.4%)
No. of Grade I, %	37 (64.9%)	46 (63.9%)	34 (63.0%)
No. of Grade II, %	18 (31.6)	21(29.2%)	17 (31.5%)
No. of Grade III, %	2 (3.5%)	5 (6.9%)	3 (5.5%)

^{a,b}: Values in the same row with different superscripts differ significantly (P<0.05).

Table 2: Follicular fluid biochemistry of lambs and ewes.

Item	Female lambs	Ewes	
Stage of oestrous cycle	Follicular phase	Follicular phase	Luteal phase
Total protein, g/dl	4.61^{b}±0.24	5.3^{a}±0.25	5.2^{a}±0.25
Urea, mg/dl	21.05^{a}±1.43	18.2^{b}±0.56	17.75^{b}±0.11
Glucose, mg/dl	33.65±1.39	34.6±0.51	35.65±0.44
Total cholesterol, mg/dl	30.67±0.31	28.0±0.47	29.67±0.78
Triglycerides, mg/dl	32.0^{a}±0.94	29.0^{b}±0.75	28.0^{b}±0.94

$^{a, b}$: Values in the same row with different superscripts differ significantly (P<0.05).

to the small follicles in addition to increase permeability of the follicle-blood barrier during follicular growth (Leroy *et al.,* 2004).

Finally, the values of total cholesterol (P>0.05) and triglycerides (P<0.05) were low in FF of ewes compared to FF of female lambs, which consistent with earlier studies (Thangavel and Nayeem 2004; Tabatabaei and Mamoei 2010; Tabatabaei *et al.,* 2010). The decreased cholesterol value in large follicle might be attributed to the conversion of cholesterol to estrogen and progesterone hormones. Additionally, the significant increase of triglycerides in smaller follicle because they might be the alternate sources of energy for follicle cells in addition to triglycerides did not pass through the follicular membrane (Harlow *et al*., 1987; Grummer *et al.,* 1988).

The continuous needs of oocyte and FF are required for *in vitro* embryo production concerning development of oocyte maturation, fertilization and culture (Mohammed *et al*., 2005; 2019a,b; Al Zeidi *et al.,* 2022a,b; AlJubran *et al*., 2023; Mohammed *et al.,* 2024a,b). It has been found that follicular fluid obtained from large follicles or after LH surge and supplemented to maturation medium (20-50%) were found to increase oocyte maturation and embryo development (Mohammed *et al*., 2005; Spacek and Carnevale, 2018). Furthermore, supernatant from cultured cumulus-granulosa cells and follicular fluid were found to improve *in vitro* maturation in patients with polycystic ovarian syndrome (POS) (Madkour *et al.,* 2018).

CONCLUSION

The evaluation of ovarian tissues, oocytes recovery and follicular fluid indicated the availability of cumulus-enclosed germinal vesicle oocytes and follicular fluid. The knowledge of the follicular fluid biochemical composition can provide useful information about the requirements of oocyte maturation for formulating suitable maturation media in lambs and ewes. Further studies are required for investigating oocyte quality, follicular fluid biochemistry and gene expression simultaneously and their relation with further embryo development.

ACKNOWLEDGEMENT

The author acknowledge the Deanship of Scientific Research, King Faisal University, Saudi Arabia for funding (GRANT 5929).

Conflicts of interest

There is no conflict of interest for authors to declare.

REFERENCES

Al-Zeidi, R., Al Masruri, H., Al Mufarji, A., Al-Hassan Mohammed, A.A. (2022a). Role of cumulus cells and follicular fluid on oocyte maturation and developmental competence of embryos: Intact and reconstructed oocytes. Adv. Anim. Vet. Sci. 10(6): 1219-1226.

Al-Zeidi, R., Al Masruri, H., Al Mufarji, A., Mohammed, A.A., Mohammed, H. (2022b). Changes in nucleus and cytoplast during oocyte maturation: Involvement in embryo production. Adv. Anim. Vet. Sci. 10(9): 2081-2089.

Aljubran, S., Al-Suwaiegh, S., Alyousef, Y., Alhajri, S., Alghareeb, M., Mohammed, A.A. (2023). Roles of assisted reproductive techniques in mammals: Developmental competence of oocytes and embryos. Adv. Anim. Vet. Sci. 11(2): 252-263.

Aller, J.F., Callejas, S.S., Alberio, R.H. (2013). Biochemical and steroid concentrations in follicular fluid and blood plasma in different follicular waves of the estrous cycle from normal and superovulated beef cows. Anim. Reprod. Sci. 142: 113-120.

Azari-Dolatabad, N., Raes, A., Pavani, K.C., Asaadi, A., Angel-Velez, D., Damme, P.V., Leroy, L.M.R., Van Soom, A., Pascottini, O.B. (2021). Follicular fluid during individual oocyte maturation enhances cumulus expansion and improves embryo development and quality in a dose-specific manner. Theriogenol. 166: 38-45.

Bartlewski, P.M., Baby, T.E., Giffin, J.L. (2011). Reproductive cycles in sheep. Anim Reprod Sci.124(3-4): 259-268.

Cerri, R.L.A, Rutigliano, H.M., Chebel, R.C. and Santos, J.E.P. (2009). Period of dominance of the ovulatory follicle influences embryo quality in lactating dairy cows. Reprod. 137(5): 813-823.

Contreras-Solís, I.M. Soto-Heras, C.S., Roura, M., Paramio, M.T., Izquierdo, D. (2021). Effect of follicle size on hormonal status of follicular fluid, oocyte ATP content and *in vitro* embryo production in prepubertal sheep. Domest. Anim. Endocrinol. 75: 106582.

Cushman, R.A., Allan, M.F., Kuehn, L.A., Snelling, W.M., Cupp, A.S., Freetly, H.C. (2009). Evaluation of antral follicle count and ovarian morphology in crossbred beef cows: Investigation of influence of stage of the estrous cycle, age and birth weight J. Anim Sci. 87: 1971-1980.

Deka, S.S., Kalita, D.J., Sarma, S., Dutta, D.J. (2014). Some biochemical constituents in follicular fluid of indigenous cows of Assam. Veterinary World 7(11): 976-979.

FAO, (2021). Characterization and assessment of small-scale livestock production, husbandry, health and technology practices in the Kingdom of Saudi Arabia. LIV/051/2021/1.

Fields, M.J., Fields, P.A. (1996). Morphological characteristics of the bovine corpus luteum during the estrous cycle and pregnancy. Theriogenol. 45: 1295-1325.

Fiscus, J., Fraison, E., Renault, L., Salle, B., Panthu, B., Elsa Labrune, E. (2023). Metabolic signature of follicular fluid to understand infertility-related diseases: A narrative review. Reprod. BioMed. Online. 2023: 103762.

Gabryś, J., Kij-Mitka, B., Sawicki, S., Kochan, J., Nowak, A., Łojko, J., Karnas, E., Bugno-Poniewierska, M. (2022). Extracellular vesicles from follicular fluid may improve the nuclear maturation rate of *in vitro* matured mare oocytes. Theriogenol. 188: 116-124.

Ganguli, G., Indra, A., Gupta, P. (1998). Suitability of the follicular oocytes obtained from slaughtered buffalo ovaries and assessment of their nuclear maturation. Buffalo J. 2: 217-227.

GAS, (2021). General Authority of Statistics, http://www.stats.gov.sa.

Gordon, I. (2003). Recovering the bovine oocyte. Establishing Pregnancies with IVP Embryos. Laboratory Production of Cattle Embryos (2nd). 30379-111321.

Grummer, R.R., Carroll, D.J., (1988). A review of lipoprotein cholesterol metabolism: importance to ovarian function. J. Anim Sci. 66: 3160-3173.

Harlow, C.R., Winston, R.M.L., Margara, R.A., Hillier, S.G. (1987). Gonadotrophic control of human granulosa cell glycolysis. Hum. Reprod. 2: 649-653.

Hashemitabar, M., Bahmanzadeh, M., Mostafaie, A., Orazizadeh, M., Farimani, M., Nikbakht, R. (2014). A proteomic analysis of human follicular fluid: comparison between younger and older women with normal FSH levels. Int. J. Mol. Sci. 15(10): 17518-17540.

Kaulfuss, K.H., Richter, A., Schulz, J. (1994). Pattern of ovarian follicle growth during the oestrous cycle in Mutton merino sheep monitored by laparoscopy. Reprod. Domestic Anim. 29: 22-37.

Leroy, J.L.M.R., Vanholder, T., Delanghe, J.R., Opsomer, G., VanSoom, A., Bols, P.E.J., de Kruiff, A. (2004). Metabolite and ionic composition of FF from different-sized follicles and their relationship to serum concentrations in dairy cows. Anim. Reprod. Sci. 80: 201-211.

Lopes, J.S., Canha-Gouveia, A., París-Oller, E., Coy, P. (2019). Supplementation of bovine follicular fluid during *in vitro* maturation increases oocyte cumulus expansion, blastocyst developmental kinetics and blastocyst cell number. Theriogenol. 126: 222-229.

Madkour, A., Bouamoud, N., Kaarouch, I., Louanjli, N., Saadani, B., Assou, S., Aboulmaouahib, S., Sefrioui, O., Amzazi, S., Copin, H., Benkhalifa, M. (2018). Follicular fluid and supernatant from cultured cumulus-granulosa cells improve *in vitro* maturation in patients with polycystic ovarian syndrome. Fertil. Steril. 110(4): 710-719.

Mohammed, A.A., Abd El-Hafiz ,G.A., Ziyadah, H.M.S. (2011). Effect of dietary urea level on biochemical composition of follicular fluid in relation to follicle size and estrous stage in Saidi ewes. Theriogenol. Insights. 2011: 33-44.

Mohammed, A.A., Abd El- Hafiz, G.A., Ziyadah, H.M.S. (2012). Effect of dietary urea on ovarian structures in Saidi ewes during follicular and luteal phases. Egyptian J. Anim. Prod. 49(1): 29-35.

Mohammed, A.A., AlGherair, I., Al-Suwaiegh, S., Al-Khamis, S., Alessa, F., Al-Madni, A., Al-Ghamdi, A. (2024a). Effects of nutritive and non-nutritive feed supplements on feed utilization, growth and Reproductive Performances in Mammals. Indian J. Anim. Res. 10.18805/IJAR.BF-1744.

Mohammed, A.A., Al-Suwaiegh, S., AlGherair, I., Al-Khamis, S., Alessa, F., Al-Awaid, S., Alhujaili, W.F., Mohammed, A., Mohammed, A. (2024b). The potential impacts of antioxidant micronutrients on productive and reproductive performances of Mammalian species during stressful conditions. Ind. J. Anim. Res. (Accepted).

Mohammed, A.A., Al-Suwaiegh, S., Al-Shaheen, T. (2019a). Effects of follicular fluid components on oocyte maturation and embryo development *in vivo* and *in vitro*. Adv. Anim. Vet. Sci. 7(5): 346-355.

Mohammed, A.A., Al-Suwaiegh, S., Al-Shaheen, T. (2019b). Changes of follicular fluid composition during estrous cycle: The effects on oocyte maturation and embryo development *in vitro*. Ind. J. Anim. Res. 54(7): 797-804. doi: 10.18805/ijar.B-1030.

Mohammed, A.A., Karasiewicz, J., Papis, K., Modlinski, J.A. (2005). Oocyte maturation in the presence of randomly pooled follicular fluid increases bovine blastocyst yield *in vitro*. J. Anim. Feed. Sci., 14: 501-512.

Mohammed, A.A., Kassab, A.Y. (2015). Metabolic changes in blood and ovarian follicular fluid in baladi goats as affected by storage time duration. Egyptian J. Anim. Prod. 52(1): 47-54.

Mohammed, A.A., Ziyad, H.M., Abd, El-Hafiz, G.A. (2011). Changes of follicular fluid composition in relation to dietary urea level and follicle size during follicular and luteal phases in Saidi Ewes. Theriogenol. Insight. 1(1): 31-42.

Mohammed, A.A. (2011). Total protein, urea, glucose, triglycerides and cholesterol concentrations of ruminant follicular fluid in relation to follicle size and estrous stage. African J. Anim. Biomed. Sci. 6(1): 127-132.

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: 100743.

Murasawa, M., Takahashi, T., Nishimoto, H., Yamamoto, S., Hamano, S. and Tetsuka, M. (2005). Relationship between ovarian weight and follicular population in heifers. J. Reprod. Dev. 51: 689-693.

Niribili, R., Jeyakumar, S., Kumaresan, A., Lavanya, M., Sinha, M.K., Kausik, M., Kamaraj Elango, K., Patil, S., Allu, T., Veerappa, V.G., Ayyasamy Manimaran, A., Das, D.N., Bhuyan, M., Ramesha, K.P. (2023). Prolonged follicular dominance is associated with dysregulated proteomic profile of the follicular fluid in Bos indicus cows. Theriogenol. 213: 34-42.

Osman, A.M., Shehata, S.H. (2005). Effect of seasons on ovarian morphology and oocytes quality in slaughtered buffaloes. Assiut Vet. Med. J. 51(105): 314-330.

Ryu, S.A., Baek, S., Kim, K.C., Lee, E.S., Lee, S.T. (2023). Effects of cumulus cells on the *in vitro* cytoplasmic maturation of immature oocytes in pigs. Theriogenol. 206: 133-139.

Sarwar, Z., Saad, M., Saleem, M., Husnain, A., Riaz, A., Ahmad, N. (2020). Effect of follicle size on oocytes recovery rate, quality and *in vitro* developmental competence in *Bos indicus* cows. Anim. Reprod. 17(3): e20200011.

SAS, (1998). SAS User's Guide: Basics. Statistical Analysis System Institute, Inc., 2008. Cary, NC, USA.

Senosy, W., Kassab, A.Y., Mohammed, A.A. (2017). Effects of feeding green microalgae on ovarian activity, reproductive hormones and metabolic parameters of Boer goats in arid subtropics. Theriogenol. 96: 16-22.

Spacek, S.G., Carnevale, E.M. (2018). Impact of equine and bovine oocyte maturation in follicular fluid from young and old mares on embryo production *in vitro*. J. Equine. Vet. Sci. 68: 94-100.

Stamperna, K., Giannoulis, T., Nanas, I., Kalemkeridou, M., Dadouli, K., Moutou, K., Amiridis, G.S., Eleni Dovolou, E. (2020). Short term temperature elevation during IVM affects embryo yield and alters gene expression pattern in oocytes, cumulus cells and blastocysts in cattle. Theriogenol. 156: 36-45.

Tabatabaei, S. Mamoei, M. (2010). Biochemical composition of blood plasma and follicular fluid in relation to follicular size in buffalo. Comp. Clin. Pathol. doi: 10.1007/s00580-010-1014-5.

Tabatabaei, S., Mamoei, M., Aghaei, A. (2010). Dynamics of ovarian follicular fluid in cattle. Comp. Clin. Pathol. doi: 10.1007/s00580-010-1038-x.

Thangavel, A., Nayeem, M. (2004). Studies on certain biochemical profile of the buffalo follicular fluid. Ind. Vet. J. 81: 25-27.

Walker, S.K., Kelly, J.M., Swinbourne, A.M.S., Weaver, A.C., Kleemann, D.O. (2023). Ovarian follicle dynamics in ewes treated with intra-vaginal progesterone pessaries. 1. Follicle waves and parameters of the estrous cycle. Theriogenol. 197: 31-36.