Egypt. J. Vet. Sci. **Vol. 56,** No. 6, pp. 1247-1254 (2025)

Egyptian Journal of Veterinary Sciences

https://ejvs.journals.ekb.eg/

Plasma Metabolomics in Mice After Treatment by Structure Nano-Lipid SNL PGF2α and PMSG during Synchronization and Superovulation Protocol in Mice

Buthina A. Abdullaha[1]* and Mohanad A. Al-Bayati[2]

[1]*Physiology and Pharmacology Department, Veterinary Medicine Collage, University of Tikrit, Iraq.*

[2] *Physiology and Pharmacology Department, Veterinary Medicine Collage, University of Baghdad, Iraq.*

Abstract

METABOLOMICS is the study of metabolites, small molecule intermediates, and metabolites. We report the metabolomics in the plasma of mice administered SNL PGF2α and PMSG during synchronized and super ovulated protocols. Groups of 3 female Albino mice were each treated first group PGF2α 14.7 µg\kg BW. and PMSG 8.3 IU\kg BW. while second group SNL PGF2α 6.6 µg\kg BW. and PMSG 8.3 IU\kg BW. and, there group. The control group received no treatment. Metabolites in plasma were extracted with methanol-chloroform-water and identified by gas chromatography-mass spectrometry (GC Mass). The remarkable markers of candidates groups of plasma provide 221 total number in conventional were 76, in control 75 metabolomics, while in SNL 72 metabolomics when comparing between the groups in the conventional group higher than other groups.

Keywords: Metabolomics, Synchronization, Superovulation, SNL, GC Mass.

Introduction

Metabolomics It means the study of chemical reaction that is related to the metabolites or it's mean the systematic detection of the sole chemical fingerprints of definite cellular activities, which detect the molecules of metabolite profiles. The metabolome represents the detection of all metabolites in cell, tissue, organ, or organism, which are the final or intermediate molecules of cellular activities genotype and xenobiotics (environmental factors, diet and drugs) control the metabolism of the phenotype [1]. Metabolomics profiling of follicular fluid from women who have been successful or unsuccessful in infertility treatment may give rise to biomarkers that indicated the likelihood of success of the treatment [2, 3]. In order to induce estrus or manipulate the estrus cycle to put a significant portion of a group of females into estrus at a specific moment, this is known as synchronization of estrus. Synchronizing oestrus in animals is an effective way to maximize the use of time and labor required to detect standing estrus in cattle and sheep [4, 5, 6]. Also, by using estrous synchronization more females animals can potentially conceive and become pregnant early in the breeding season with no decrease the fertility [7, 8]. While Superovulation allows producing a greater number of oocytes and embryos than the normal cycle in female animals Ten or more live oocytes can be collected in each estrus from superovulated cows and heifers [9]. Metabolomics is a new strategy broadly used in the reproductive turnovers for both basic physiology and clinical manoeuvres [10, 11]. This can assess in the discovery of potential biomarkers for the diagnosis of PGF2α and PMSG hormones deleterious effect on the ovarian function as compared to formulary Nano Lipid PGF2α and PMSG entrapment protecting effect.

Material and Methods

Preparation of Nano lipid PGF2α

*Corresponding author: Buthina A. Abdullaha, E-mail: buthinahameed@tu.edu.iq, Tel.: +964 770 171 5129
(Received 20/04/2024, accepted 11/06/2024)
DOI: 10.21608/EJVS.2024.282292.1995

Nanolipids were prepared using a solvent diffusion method; the preparation consisted of the following.

The lipid phase consists of two forms: 1. Lipid form: The lipid state was formed by dissolving 100 mg stearic acid in 100 mg glyceryl monostearate and dispersed with 2 ml castor oil at 800 rpm by vortexing at 1500 rpm. Spin for 30 minutes to form a lipid dispersion, 2. Dissolved form: The formed lipid phase is dissolved in 100 mg of phosphoric acid, the amount of hormone required to load and disperse is 800 rpm for 30 minutes. The dissolved form was then added to the lipid form and dispersed at 800 rpm for 1 h and stored in a refrigerator at 8 °C overnight until use. Mix by stirring at 800 rpm for 30 minutes before use [12]. The collection and filtration of supernatant was through 0.2 µm filter membranes The hormone was measured by UV-Absorbance spectrophotometer of supernatants and SNL.

Experimental design

Forty-five (45) mice are mentioned designed form and group as followed:

1- Control :- group without treatment(15 mice)

2-Conventional $PGF_2\alpha$ and PMSG (15 mice)

3- SNL $PGF_2\alpha$ and PMSG (15 mice)

After inducing the protocol at 7 AM (Fig.1) Treated by conventional $PGF_2\alpha$ 14.7 µg\kg BW. and PMSG 8.3 IU\kg BW. and SNL $PGF_2\alpha$ 6.6 µg\kg BW. and PMSG 8.3 IU\kg BW. (According to the effective dose, ED_{50}).

The day after complication of estrous cycle synchronization and superovulation protocol isolate the blood from the mice and examination the plasma by gas chromatography mass spectrophotometry GC-MS analysis

Sample preparation for gas chromatography mass

One ml of dry propolis extract was reaction with (15 µl) pyridine and (one hundred µl) N-Methyl-N-tri-methyl-silyl-trifluoroacetamide (MSTFA) included in a closed glass tube for (30 min at 60°C) to preparation samples for GC mass. Sample size of (one µl) were injection and analysis by GC-M spectrophotometry.

Gas chromatography- mass spectrometry analysis:

GC-MS analysis was verification by automated pyrolysis GC-MS (py-GCMS) type Shimadzu GCMS-QP 2010 combined with electron impact ionization (70 eV) to identify the various complexes appearing in it. Approximated (one µg) of propolis extract prepared for GM was injection by (ten µL) syringe into the quartz chamber of the pyrolysis unit and then heated in an oxygen-free environment at 400 °C. The temperature injector was 280° C and the temperature of the interface was 230 ° C and the MS scan range was 35 to 450 atomic mass units (AMU) .

The chromatographic column used for analysis is an RTX-5MS capillary column with a length of 60 m, an inner diameter of 0.25 mm, and a thickness of 0.25 µm, containing 5% biphenyl and 95% methylpolysiloxane. The oven temperature program was initially set to 50°C for the first 6 minutes and then increased to 280°C for 21 minutes. Helium was used as carrier gas with a flow rate of 20 ml/min. Mass spectrometry was performed with an ion source temperature of 200 °C, electron impact ionization of 70 eV, and a mass range (BM) of 40 to 600 m/z.

Results and Discussion

Metabolomics profile

The Fig. (2) heat map showing the metabolomics profile in female mice groups SNL PGF2α-PMSG, Conventional PGF2α-PMSG, and control as a Synchronization percent value superovulation technique.

The heat map exhibited several metabolome integrations in Fig. (3) the number of metabolomics detected from groups at different time figures showed total number in conventional was 76, in control 75 metabolomics, while in SNL 72 metabolomics. When comparing between groups, The diagnostic markers of metabolomes are concluded in the schematic diagram. The markers are dosed while significantly appearing or disappearing, and increasing or decreasing metabolome fractions that are statistically significant ($p \leq 0.05$) based on group marker differentiation between treated groups: control, conventional and SNL PGF2α-PMSG of Palmitic acid under estrous synchronization and superovulation, SNL PGF2α-PMSG and conventional cis-Vaccenic and SNL PgF2α – PMSG protocolled mice oleic acid, Ecosanoid, and decosanoid.

Detection of Metabolomics at different times: In Fig. (4) showed metabolomics number essential and inessential between groups at different times in plasma mice it was a common largest number between the control group and SNL group.

The metabolomics counts were counted in relative levels to control areas of each metabolite to compare the amount change in metabolite at different times, there were a lot of numbers common between the control group and conventional, SNL. the ratio of the numbers common in SNL group was low than control group (Fig.5).

The metabolomics analysis can enhance better identification of the real-time status of biological systems during a physiological or illness process and provide useful indicators of those processes as what happens during reproduction. these

metabolites, which were the end-products or essential components of a metabolic pathway, can potentiate the diagnosis of any abnormalities during treatment. This systematic study has revealed data and inconsistencies in the literature regarding metabolomics studies in the field of the protocolled synchronization superovulation that was accommodated with the biological clock as chronothesy [13].

The majority of the peaks of the chromatograms were identified as an endogenous compounds by GC-Mass such as amino acid, lipid, and, carboxylic acids that are well known for their participation in multiple biochemical processes, especially in energy and lipid metabolism [14].

The current results showed variations in total compounds. The total screen primary in terminated metabolite involved in 221 plasma compounds, conventional 76, control 75 metaboloms, and SNL PGF2α-PMSGin valued 72 metaboloms. The metaboloms number comparing between groups control and conventional at chronotherapy set times the result of chrontheraputic at 19:00 was, showed higher efficiency at all treated groups, and then followed at 7:00 in the conventional and SNL PGF2α-PMSG, while a number of metaboloms at 13:00 is lower in all chronotheraputic groups.

The result synchronization-superovulation group in conventional and SNL was the best outcome resulting evaluated parameters at 19:00, the ideal marker for identification compatible function of synchronization-superovulation SNL PGF2α-PMSG within protocol without generalized of changes In the body as compared with control for marker chose changes, hence the lower numbers minimized that physiological interaction approved with protocol of main the sequences reaction may be due decreased the generalized stress reduced feedback in physiological gain that may be attributed to SNL may be target ether aimed organ with less affinity to other generalized effect.

The metabolomics analysis of plasma after the induction of estrus synchronization and superovulation by PGF2α–PMSG protocol of our study revealed to several biomarker metabolites, including Palmatic acid in The three groups control, conventional and SNL, while in conventional group revealed Cis –vaccenic, As for the SNL group, three types of metabolic results were discovered Oleic acid, Palmitic acid, Eicosanoids, and, Docosanoids.

The results of this study significantly indicated the appearance of fatty acid in metabolic profiles of synchronization and superovulation protocol in female mice. Includes Oleic acid, Palmitic acid, eicosanoids and, docosanoids generally Fatty acids were known to be critically important in multiple biological functions. Fatty acids of follicular fluid metabolome, an important drive microenvironment for the development of oocytes, these fatty acids accumulate as lipid droplets during oocyte growth, and fatty acid oxidation can generate a high number of ATP molecules making these molecules good candidates for energy provision during oocyte maturation [15]. Fatty acid may be having good indicative marker for superovulation and their tonicity of fatty individual give marker of ovarian follicle development. Perhaps fatty acids are the metabolites produced by the production of steroid hormones. The steroid hormones were intensity during SNL PGF2α-PMSG protocol due to turnover effect and targeted ovarian cycle.

The steroid estrogen synthesis metabolite fatty acid affecting on cells membrane stability and fluidity aromatase cytochrome P450- NADPH-cytochrome P450 reductase system [16]. The accumulation of these fatty acids as lipid droplets during oocyte development makes these molecules good candidates for providing energy during oocyte maturation [17]. The fatty acids metabolite within the oocytes increases dramatically during the maturation of the oocyte complex [18].

The results showed that Palmitic acid was the biomarker of the synchronization of superovulation in all treated groups. Palmitic acid is generated via fatty acid oxidation in mitochondria used to generate ATP. An excessive amount of Palmitic acid induces endoplasmic reticulum stress and mitochondrial dysfunction causes lipid accumulation in cell [13]. The Palmitic acid found all chronotheraputic groups protected estrus synchronization and superovulation. Palmitic acid is one of the most common fatty acids in animal and human follicular fluid and blood serum [1].

The ovarian dominant activity was displayed increase Palmate acid may be due to improved estrogenic metabolism as catabolism. Palmate acid may be result from the metabolom activity of follicles depending on the activity of estrogen in granulosa cell proliferation. This fact was coincided with result of granolas cell number in Figure 4.12. Also, palmate acid may be due to referring successful on oocyte quality, As well as the

fertilization and development of the blastocyst that increases the chance of palmate acid marker [19, 20]. While the results showed that cis-vaccenic acid was the biomarker of the synchronization superovulation for conventional group. cis-vaccenic acid, is an omega-7 fatty acid, name is (11E)-11-octadecenoic acid, vaccenic acid led to exhibited in lowered total cholesterol, lowered LDL holesterol and lower triglyceride levels [21]. Hence activity of Cis-Vaccenic acid may be found during cholesterol-steroid of metabolim. While the results showed that eicosanoids and docosanoids was the metabolomics compounds of the synchronization superovulation groups. Poly fatty acid as a phospholipid drive from arachedonic acid mainly found plasma membrane in corporation with other fatty acid. The changes between C20-22 may be the polyunsaturated fatty acid may be presumably changes composition during metabolom turnover in ovarian cellular ingredient give a chance of promoting marker as follicular development [22].

Polyunsaturated fatty acid eicosapentaenoic, docosahexaenoic acids poly unsaturated fatty acid act as mediators in a series of processes in several reproductive tissues, including the fluidity of cell membrane [23]. The Polyunsaturated fatty acid was represent eicosapentaenoic, docosahexaenoic acids marker for SNL PGF2α-PMSG protocol as compound with conventional. Polyunsaturated fatty acids, such as docosahexaenoic acid and eicosapentaenoic acid can inhibit inflammation by regulating the activity of inflammatory signaling pathways and influencing the production of lipid mediators [24]. One of the most important steps of oocyte maturation is meiotic resumption, and both granulosa/cumulus cells together with poly unsaturated fatty acid metabolite play a primary role dominance effect [25]. Cellular Tran forming of Oocyte and embryonic survival high concentrations of eicosapentaenoic and docosahexaenoic acids may reduce the synthesis of PGF2α and delay regression of the corpus luteum, improving Oocyte and embryo survival and herd fertility [26]. As well as increasing implantation and pregnancy rates. Oleic acid was chosen a marker of SNL PGF2α-PMSG protocol. The Oleic acid presumably increases lipid storage in lipid droplets and improves the efficiency of Oocyte development and related to the maturation of Oocyte. The Oocytes were able to incorporate Oleic acid into their own phospholipids; for their metabolism or for in cellular membranes stability utilities of Ova. The Oocyte released through the follicular fluid directly affect the quality of the oocyte and then embryo and thus the fertilization rates these well owed in result pregnancy rate, and, fetus number [27].

Conclusion

After conducting protocol Estrous cycle synchronization and superovulation (SO) protocol managed by PGF2α – PMSG hormone in mature female mice, and examining the plasma with GC Mass, The metabolome was shown to be helpful in the current investigation in identifying a class of biomarkers that correlate to the protocol for superovulation (SO) and estrous cycle synchronization for each group.

Conflict of interest

The authors declare no conflicts of interest associated with this manuscript.

Acknowledgments

The authors are very grateful for the support received from the department of Physiology, Pharmacology and Biochemistry department, College of Veterinary Medicine, University of Baghdad.

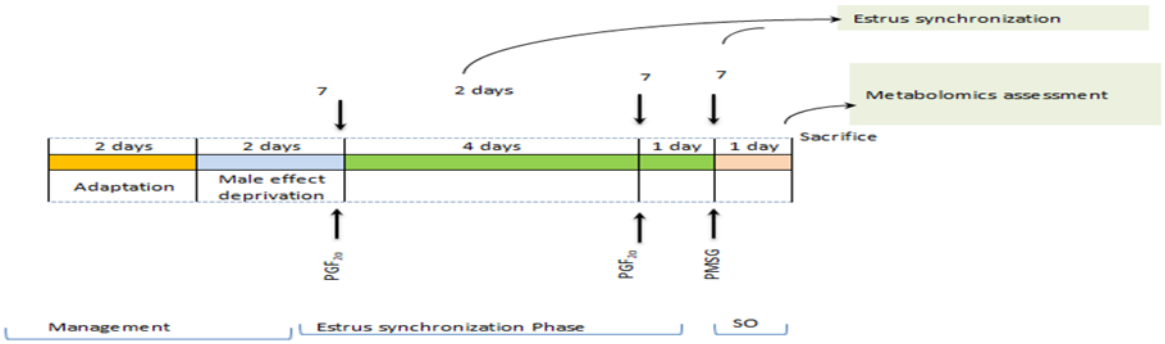

Fig. 1. Protocol synchronization of Estrous and superovulation by using PGF2α and PMSG in mice.

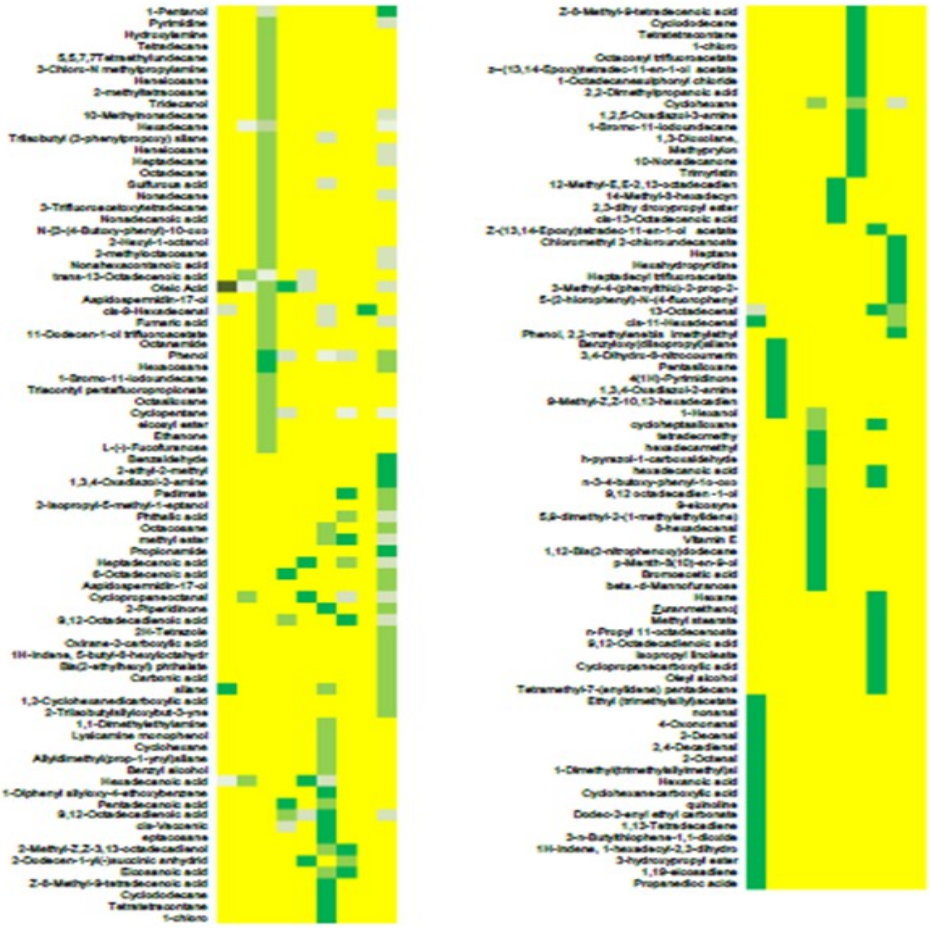

Fig.2. Heat map as graphical visualization of MetFlow and Metabo Analyst (5.0 version) showed different metabolome and colure as values are based on the p≤0.05 the demo-rich metabolome the darkness color indicates the it is loaded metabolite values and lightness indicates the metabolites in uploaded data which denoted to the different levels.

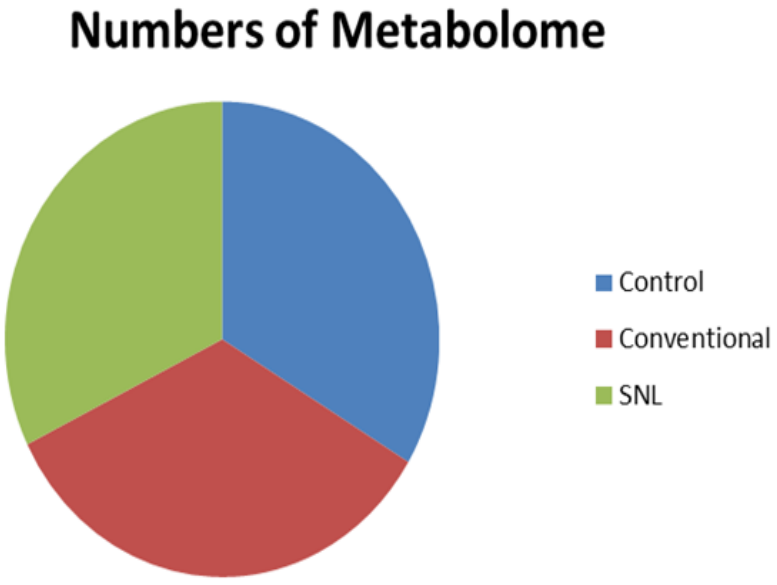

Fig. 3. Number of metabolomics detected

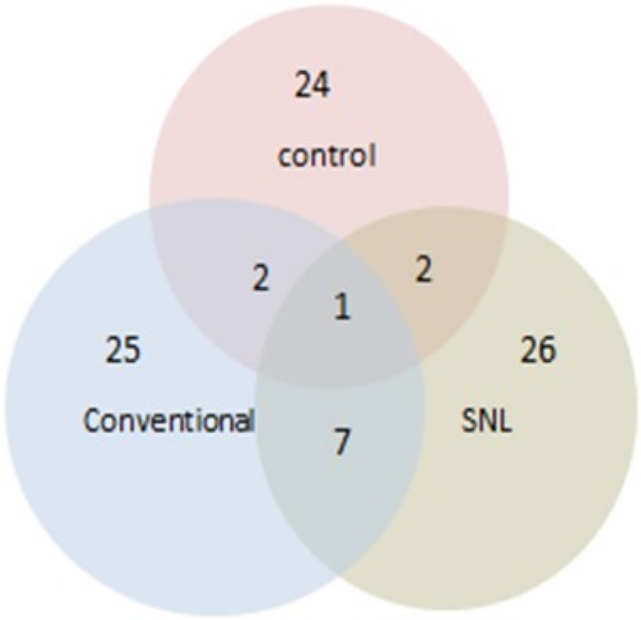

Fig. 4. Number of metabolomics detected essential and unessential

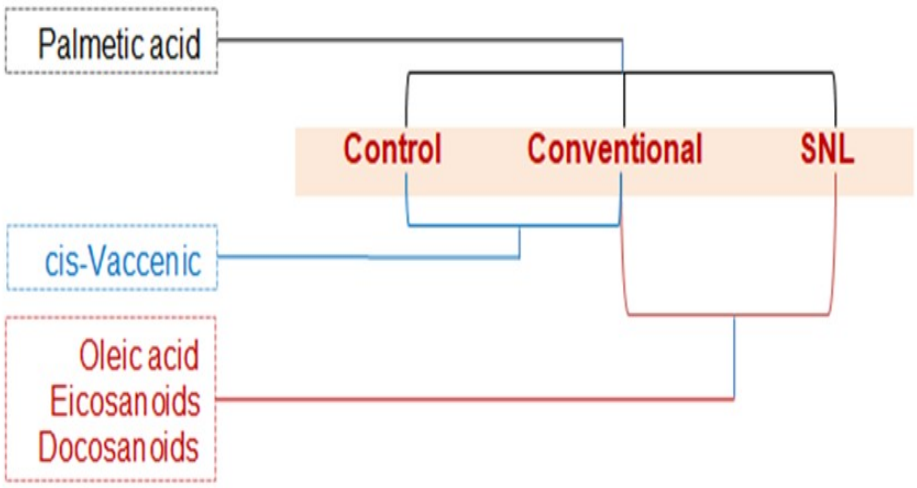

Fig. 5. Schematic plan of metabolomics profiling map biomarkers in protocolled estrous synchronization and superovulation in SNL and conventionally treated mice.

References

1. Yang, Z. and Marotta, F. Pharmacometabolomics in Drug Discovery & Development: Applications and Challenges , Metabolomics. 2153-0769. *JOM an Open Access J*., **2**(5), 1000-122 (2012).
2. Di Leo, A., Claudino, W., Colangiuli, D., Bessi, S., Pestrin, M. and Biganzoli, L. New strategies to identify molecular markers predicting chemotherapy activity and toxicity in breast cancer. *Annals of Oncology*., **18** (12), xii8–xii14 (2007).
3. Alrudainy, A.j. & Jumaa, Q.S. Effect Of Magnitized Water On Common Carp Cyprinus Carpio Rearing In Different Salinity Concentrations. *Iraqi Journal Of Agricultural Sciences*, **47**(2), 646-655(2016).
4. Abd, A. A. and Ibrahim, S. N. Diagnosis of Pregnancy in Iraqi Awassi Ewes Through Progesterone Hormone Measurement and Ultrasonography Following Induction of Fertile Estrus with Sulpiride. *Egyptian Journal of Veterinary Sciences,* **55**(4), 945-953 (2024).
5. Ali, A. A. and Najlaa, S. I. Induction of oestrus by Sulpiride and measurement of estrogen hormone in Iraqi AwassiEwes during the out of breeding season. *The Indian Veterinary Journal*, **100**(12), 15-18(2023).
6. Jumma, Q. Detection of ESBL E.coli That Carried STX1 and STX2 Form Common Carp (Cyprinus carpio) in Salhaldeen Province. *Egyptian Journal of Veterinary Sciences*, **55**(4), 1165-1170(2024).
7. Pursley, J. R. Deep uterine horn AI improves fertility of lactating dairy cows. *J. Dairy Sci.,* **87** (1),372(Abstract) (2004).
8. Younis, L.S., Abid, A.A. and Rasheed, S.T. Effect Of Polymorphism G(129)R In Growth Differentiation Factor 9 Gene (Gdf9) On Awassi Ewes That Breed Out Of Season. *Biochemical & Cellular Archives*, **2**, 69 – 73(2019).
9. Ayasan, T. and Karakazak, E. Nutrition of Donor Cows. *Kafkas Univ. Vet. Fak. Derg.,* **16**, 523-530 (1994).
10. Bo, G.A., Hockley, D.K., Nasser, L.F. and Mapletoft, R.J. Superovulatory response to a single subcutaneous injection of Folltropin-V in beef catle. *Theriogenology*, **42**, 963-975(2010).
11. Yang, X. Z., Dou, S., Wang, Y.C., Long, H.Y., Xiong, M.H., Mao, C.Q., Yao, Y.D. and Wang, J. Single-step assemblyof cationic lipid–polymer hybrid nanoparticles for systemic delivery of siRNA. *ACS Nano,* **6**, 4955–4965 (2012).
12. Hussein, A. & Jumma, Q.S. Diagnosis of Infectious Bronchitis Infection in Broiler Chicken Farms in Salah Al-Din Governorate. *Egyptian Journal of Veterinary Sciences,* **55**(6), 1619-1626(2024).
13. Lee, J., Hong, S.W., Park, S.E., Rhee, E.J., Park, C.Y., Oh, K.W., Park, S.W. and Lee, W.Y. Exendin-4 inhibits the expression of SEPP1 and Fetuin-A via improvement of palmitic acid-induced endoplasmic reticulum stress by AMPK. *Endocrinol Metab. Seoul*., **30**, 177−184 (2015).
14. Alshumary, H., Jumma, Q., Khorsheed, H., AlKaisi, B. Assessment of the toxic effect of environmental pollution by 2 ,3 ,7 ,8 - Tetrachlorodibenzo-p-dioxin (TCDD) on the female reproductive system by pathological and biochemical assay in Albino female rats. *Egyptian Journal of Veterinary Sciences*, **55**(5), 1409-1415(2024).
15. Thapar, R. and Titus, M. Recent advances in metabolic profiling and imaging of prostate cancer metabolomics. *Current Metabolomics*., **2**, 53-69(2014).
16. Sturmey, R.G., Reis A., Leese H.J. and McEvoy, T.G. Role of fatty acids in energy provision during oocyte maturation and early embryo development.*Reproduction in Domestic Animals*, **44** (3), 50–58 (2009).
17. Conley, A. Hinshelwood M, Mammalian aromatases. *Reproduction*, **121**, 685–695(2001).
18. Santos, J., Cerri, R. and Sartori, R. Nutritional management of the donor cow. *Theriogenology,* **69**(1), 88–97(2008).
19. Nobuhiko, I., Koumei, S., Takehito, K. and Hisataka, I. Palmitic acid induces ceramide accumulation, mitochondrial protein hyperacetylation, and mitochondrial dysfunction in porcine oocytes. *Biology of Reproduction*, **98**(5), 644–653 (2018).
20. Choi, W.J., Banerjee, J., Falcone, T., Bena, J., Agarwal, A. and Sharma, R.K. Oxidative stress and tumor necrosis factor-alpha-induced alterations in metaphase II mouse oocyte spindle structure. *Fertil. Steril*., **88**,1220–1231.1237 (2007).
21. Haze, S., Gozu, Y., Nakamura, S., Kohno, Y., Sawano, K., Ohta, H. and Yamazaki, K. "2-Nonenal newly found in human body odor tends to increase with aging". *J. Invest. Dermatol.,* **116** (4), 520–524(2001).
22. Mattos, R., Staples, C. and Thatcher, W. Effects of dietary fatty acids on reproduction in ruminants. *Rev Reprod.,* **5**(1), 38-45 (2000).
23. Wathes, D. C., Abayasekara, D. R. and Aitken, R. J. Polyunsaturated fatty acids in male and female reproduction. *Biology of Reproduction*., **77**(2),190-201 (2007).
24. Calder, P. C. Long chain fatty acids and gene expression in inflammation and immunity. *Current Opinion in Clinical Nutrition and Metabolic Care*., **16**(4),425–433(2013).

25. Jungheim, E.S., Louden, E.D., Chi, M.M., Frolova, A.I., Riley J.K. and Moley, K.H. Preimplantation exposure of mouse embryos to palmitic acid results in fetal growth restriction followed by catch-up growth in the. *Biology of Reproduction,* **85**, 678–683 (2011).

26. Staples, C. R. and Thatcher, W. W. Effects of fatty acids on reproduction of dairy cows. In: Garnsworthy PC, Wiseman, J. (Eds.), Recent Advances in Animal Nutrition. *Nottingham University Press. Nottingham, UK,* 229- 256. (2005).

27. Leo, A., Claudino, W., Colangiuli, D., Bessi, S., Pestrin, M. and. Biganzoli, L. New strategies to identify molecular markers predicting chemotherapy activity and toxicity in breast cancer. *Annals of Oncology*., **18** (12), xii8–xii14. (2007).

28. Jabbou, H.N., Sales, K.J., Catalano, R.D. and Norman, J.E. Inflammatory pathways in female reproductive health and disease. *Reproduction*, **138**(6),903–919 (2009).

استقلاب البلازما في الفئران بعد العلاج بواسطة النانو الدهني للبروستوكلاندين 2الفاوو مصل الفرس الحامل أثناء التزامن وبروتوكول الإباضة الفائقة في اناث الفئران

بثينة عبدالحميد عبد الله [1] و مهند عبد البياتي [2]

[1] فرع الفسلجة والادوية - كلية الطب البيطري - جامعة تكريت - العراق.

[2] قسم الفسلجة والادوية - كلية الطب البيطري - جامعة بغداد - العراق.

الخلاصة:

يقوم علم الأيض بتقييم المستقلبات والجزيئات المتوسطة الصغيرة ومنتجات التمثيل الغذائي. نقوم بالإبلاغ عن عمليات التمثيل الغذائي في بلازما الفئران التي تم إعطاؤها SNL PGF2α و PMSG أثناء بروتوكول التزامن والإباضة الفائقة. عولجت كل مجموعة من 3 إناث فئران ألبينو في المجموعة الأولى بـ 14.7 PGF2α ميكروجرام/كجم من وزن الجسم. و 8.3 PMSG وحدة دولية\كجم من وزن الجسم بينما المجموعة الثانية 6.6 SNL PGF2α ميكروجرام\كجم من وزن الجسم. و 8.3 PMSG وحدة دولية\كجم من وزن الجسم، ولم تتم معالجة مجموعة التحكم. تم استخلاص المستقلبات في البلازما في الميثانول - كلوروفورم - ماء وتم التعرف عليها بواسطة قياس الطيف الضوئي الكتلي للغاز (GC Mass). وكانت العلامات البارزة لمجموعات البلازما المرشحة توفر 211 عددا إجماليا في التقليدية كان 76، في السيطرة 75، بينما في 72 SNL استقلابيا عند المقارنة بين المجموعات في المجموعة التقليدية أعلى من المجموعات الأخرى

الكلمات المفتاحية: التمثيل الغذائي، توحيد الشبق، فرط الإباضة ، البنية النانوية الدهنية، مطياف كروماتوكرافيا الغاز-الكتلة.