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Effects of Nano-Lipid Prostaglandin $F_{2\alpha}$ on Synchronization

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ABSTRACT

Background: By modulating the estrous cycle, numerous oestrus-inducing or synchronising therapeutic hormones and medicines are accessible for pharmacological use, allowing for rapid onset of estrus in the majority of animals.

Objective: The purpose of this research is to evaluate the efficacy of Nano-Lipid containing prostaglandin F2 nanotechnology in synchronising the estrous cycle.

Material and Methods: The strategy to induce and synchronise estrus75 in 4- to 8-week-old female mice was tested in an animal investigation to establish its efficacy across three treatment groups: control, conventional, and SNLPGF2. The first group received a saline injection as a control, the second group received conventional hormone injections at 5, 10, 15, 20, and 25 IU g/kg body weight (I.P.), and the third group received SNL PGF2 hormone injections at 5, 10, 15, 20, and 25 IU g/kg body weight I.M.

Results: Nano-lipid Size The PGF2 ranged in length from 50 to 166.6 nm. whereas SNL PgF2 trapping efficiency is 84.11 (SD 5.55). As expected, autolysis and a rise in the estrus phase and an increase in the amount of estrogen were responsible for the greatest degree of synchronisation in the group that received large doses of nano-treatment**.**

Conclusion: results of this research demonstrate that Prostaglandin F2 f Nano-lipid has a high efficiency for synchronising the estrous cycle..

Keywords: Synchronization, Estrus cycle, Nano-Lipid, Prostaglandin F2α.

INTRODUCTION

The cycle of estrous indicates the cycle of reproductive. Similar to the reproductive cycle in humans, the estrous cycle consists of four phases, namely, proestrus, estrus, mesentery, and remission, and lasts for 4 to 5 days [(1)]. both LH and FSH govern the estrous cycle [(2)]. There are two distinct times in a menstrual cycle: the luteal and the follicular phases. [(3)].

The luteal phase is the period following ovulation when the corpus luteum (CL), while the follicular phase is the period following the demise of the corpus luteum until ovulation (often further designated as pro-oestrus and oestrus), luteolysis by PGF2 α[(4)]. PGF2α is the treatment of choice to induce estrus by analyzing lutein body profile [(5)]. The use of PGF2α is one of the protocol methods for synchronizing estrus by shortening the luteal phase [(6)].

The synchronization of estrus allows the offspring to be increased, due to its inhibitory effect on the reproductive cycle and without dependence on the season. It also unifies the estrus period. Hormonal products are used in the synchronization of estrus for natural mating or artificial insemination, which further improves the reproductive performance of animals.

The use of prostaglandins is an alternative method for controlling reproduction, which eliminates corpus lutum and leads to a later follicular phase with ovulation [(7)]. Nanotechnology is a drug delivery system that can increase drug permeability by increasing their solubility. As well as increasing issues of premature removal associated with small particles, regenerative medicine will play a major role in treatment in the future [(9,8)]. SLNs are solid lipids (e.g) triglycerides, complex mixtures of glycerides or waxes) and stabilized by surfactants [(10)].

The study was aimed at the reformulation and optimization of PGF2α based on the Nanostructured lipid carriers' improved protocol of the estrous cycle synchronization

MATERIAL AND METHODS

Preparation of Nano-Lipid PGF2α

Nano lipid was prepared by solvent diffusion method; the formulation consisted.

The lipid phase was comprised of two forms:

- **Lipid form**: The lipid status formed by Stearic acid 100 mg dissolved by glycerine monostearate 100 mg and dispersing 800 rpm with Castor oil 2 ml to form lipid dispersion by vortex 1500 rpm for 30 minutes.

- **Dissolving form**: At 800 rpm for 30 minutes, we loaded and disseminated the necessary quantity of hormones into the developing lipid phase and then dissolved it in 100 mg of phosphatic acid. After adding the dissolved form to the lipid form and mixing at 800 rpm for 1 hour, the mixture is chilled overnight at 8 °C before being utilised. You should stir for 30 minutes at 800 rpm before using (11). After collecting the supernatant, it was filtered via membrane filters measuring 0.2 m. UV-Absorbance spectrophotometer readings of supernatants and SNL were used to detect the hormone.

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The Standardization of SNL

Identification, shape, and size of structural Nano-lipid were investigated and standardized with parametric manoeuvres [12].

Light micrograph of Systemic Nano Lipid hormones smear, prepared and examined under a microscope; optical microscope scan and electron microscopy micrograph of Structural Nano Lipid size.

Determination of entrapment loading and efficiency

The percentage of entrapped hormones from Nano-lipid was measured after complete loading of (PMSG and PGF2α); the methodological protocol was done according to [13]. The entrapping efficiency and loading were calculated using the following equations:

EE %= Total amount-free amount (supernatant) / Total amount× 100

DL% = (Total amount of content-Amount of free content) / (Total amount of lipid) × 100

Experimental design

Seventy-five mice are mentioned in designed form and group as followed:

1. Control: 25 mice treated by normal saline.
2. Conventional PGF2: A total of 25 mice will be given doses of 5 g/kg BW, 10 g/kg BW, 15 g/kg BW, 20 g/kg BW, and 25 g/kg BW, respectively, in accordance with figure 15 of the serial can amount methodology.
3. Nano-Lipid PGF2α: 25 mice will dosed 5 µg\kg BW, 10µg\kg BW, 15 µg\kg BW, 20 µg\kg BW, 25µg\kg BW to serial can amount of protocol figure.

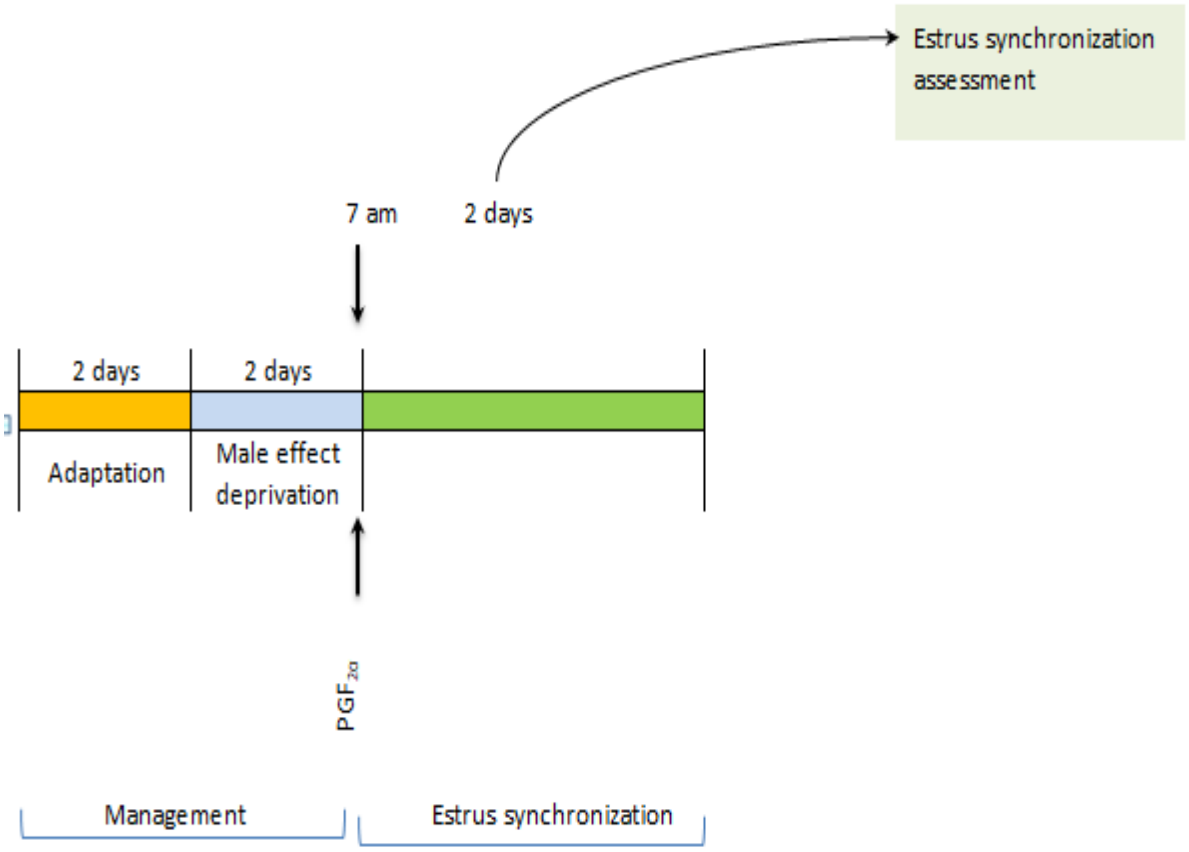


Figure (1): Estrous cycle synchronization, protocol managed by PGF2α hormone in mature female mice.

Detection of estrous cycle phase's periods

Vaginal cytology was used to differentiate between the stages of the estrous cycle (proestrus, estrus, metestrus, and diestrus). Warm, sterile, normal saline 0.85% (100 l) was used to cleanse the vaginal area. The vagina was injected with 50 l of normal saline, and then the fluid was removed and the operation was repeated 4-5 times.. Cells extracted from the vagina were stained on the slide and then dried at room temperature 25°C. Vaginal swabs were then stained with methylene blue (1%) for 1 minute and washed with distilled water twice. Vaginal cytology was assessed according to the method of Mcleanet al. [(16)] by recording the percentage of cell types (leukocytes, nuclear epithelial, and squamous keratinized cells) and the relative proportion of the cell class individually present during the four stages of the estrus cycle in the mouse and adapted to the wheels of the estrous cycle for the distribution of Cells with time recording of all phases of the estrus cycle.

Ethical consideration

Ethics for mouse care were based on the ACUC's "Guide for the Care and Utilization of Laboratory Animals in studies and teaching," and for pharmacological dosage and curative manoeuvres, Al-Bayati and Khamas's "Ethical Craft in the Use of Medicine" [(14)].

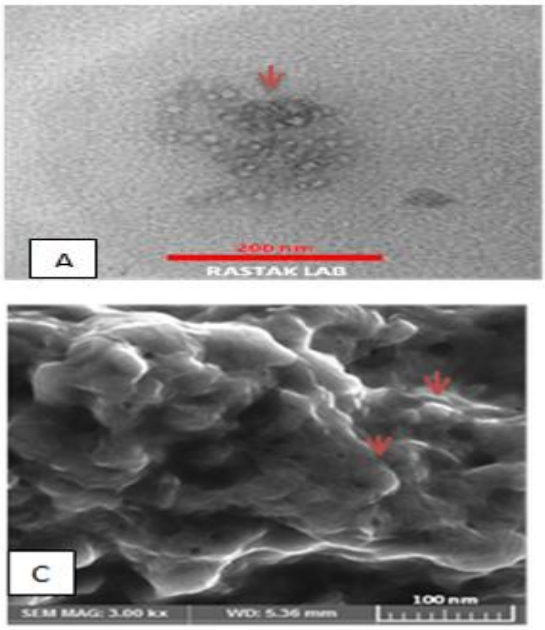
Statistical analysis

Information was gathered and coded in Microsoft Excel. The information was transferred to SPSS 20.0 (Statistical Package for the Social Sciences) for further examination. Microsoft Excel 2010 was used for the graph creation. Quantitative data was shown as means and standard deviations, whereas qualitative data was shown as frequencies and percentages of the whole.. P value ≤0.05 was considered significant.

RESULTS

Scanning and transmission electron microscope

Electron microscopy scanning and transmission images were used to determine the chemical composition and structure of Nano-Lipid hormone formulations.. A scan depiction of the Nano-Lipid was shown fine spherical separated vesicular assemblies. The transmission mode depiction image exhibited small size of the Nano-Lipid figure



Figures (2): Ultrasound and TEM: (A) Electrons are sent via a transmission medium. (C) Electron microscopy.

Entrapment efficiency and loading %

The entrapment efficiency and loading % of hormones PGF2α is mentioned in for five patches (Table 1).

The amount percentage of the amount percentage of PGF2α in the SNL was 84.11. The loading efficiency percentage of hormones PGF2α was 81.04.

Table (1): The loaded profile of SNL $PgF_{2\alpha}$ and percentile of entrapment values**.**

SNL hormones	Entrapment %	Loaded %
SNL $PgF_{2\alpha}$	84.11 ± 5.55 b	81.04 ± 6 b

N= 5 samples, Data presented ± SE of mean, SNL: structure Nano-Lipid, PGF2α: Prostaglandin F2 alpha.

Nano Size of structure Nano Lipid SNL carrying hormones PMSG and $PGF_{2\alpha}$

The particle size of Nano-metric scale was value at 50-166.6 nm of SNL PGF2α and SNL PMSG 85.7-300nm (Table 2).

Table (2): Size of SNL carrying hormones; PMSG and PGF2α**.**

Type of SNL hormones	Size of Nano-lipid nm	
	Range size	Mean ± SE
Nano-lipid Carrying PGF2α	50-166.6 b	99.8 ± 17.5 a

N= 5 samples, Data presented ± SE of mean, SNL: structure Nano-Lipid, PGF2α: Prostaglandin F2 alpha.

Estrous cycle features after PGF2 Conventional and SNL synchronisation (Syc) technique:

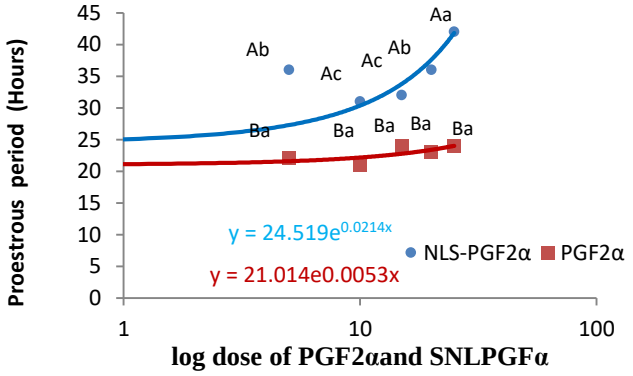
The Log dose response curve of estrous cycle period lengths post protocolled Sync exhibited in **Figure 3**. the indices of estrous cycle length analysis of phases and their intervals.

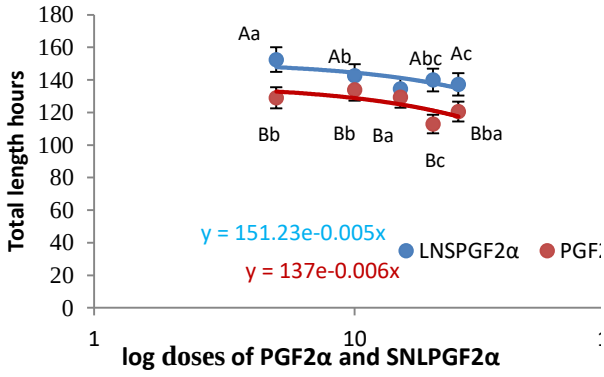
1. **Total estrous cycle period:** The depiction displayed the log dose response curve of total estrous cycle period after Sync-SupO figure showed both groups conventional and SNL were reduction ($p \leq 0.05$) in length period as fallows the doses increased.
2. **Proestrous phase length period:** The log dose response curve of pro-oestrous figure after Sync, the SNLPGF2α showed longer period significantly ($p \leq 0.05$) than conventional protocol. In otherwise the dosed 10 and 15 mg/kg Bw ip. groups were decreased periods significantly ($p \leq 0.05$),as compared with other dosed.
3. **Estrous phase length period:** Figure 3 was displayed the log dose response curve of oestrous after Sync, the SNLPGF2α showed longer estrous period significant ($p \leq 0.05$) than conventional form PGF2α.
4. **Met estrous phase length period:** The log dose response curve of Met-oestrous figure 3 after Sync, the SNLPGF2α showed Metaestrous phase conventional form PGF2α significantly ($p \leq 0.05$) faster disappeared than SNLPGF2α.
5. **Diestrous phase length period:** The log dose response curve of diestrous post Sync, the SNLPGF2α displayed shorter diestruous period significantly ($p \leq 0.05$) as compared to conventional form PGF2α the decrement diestrous period was followed the dose increment.

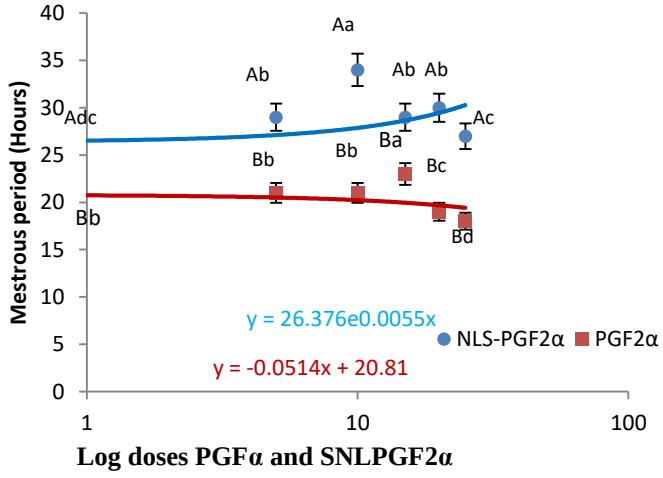
PGF2-PMSG Conventional and SNL Protocol for Postmenopausal Progesterone and Estrogen Levels

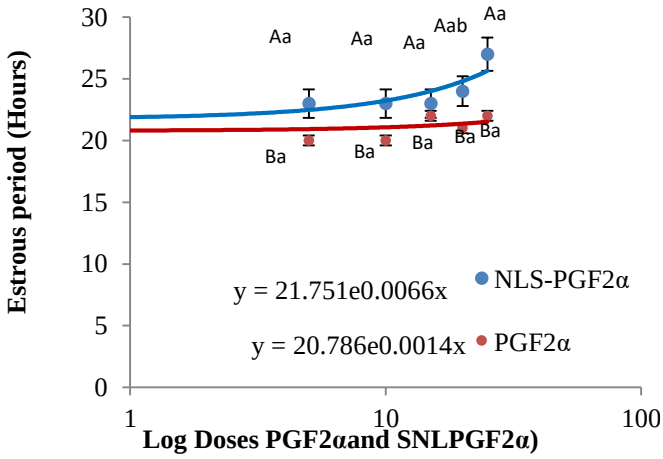
Figure 4 displayed the Log dosage response curve of level hormones (progesterone & oestrogen) after a protocolized Sync -SupO administration.):

1. **Progesterone hormone level:** This depiction displayed the log dose response curve of Following estrous cycle synchronisation, progesterone levels in both groups decreased according to the dosages given. Significantly greater Sync-SupO levels were seen in the SNL leading to the production group compared to the standard protocolled group. ($p \leq 0.05$).
2. **Oestrogen hormone level:** This depiction displayed the log dose response curve of level of hormone oestrogen after Sync-, showed both groups increment levels of hormone as the doses increase. The SNL protocolled group displayed higher level than conventional protocolled group of Sync significantly ($p \leq 0.05$) (**Figure 4**).









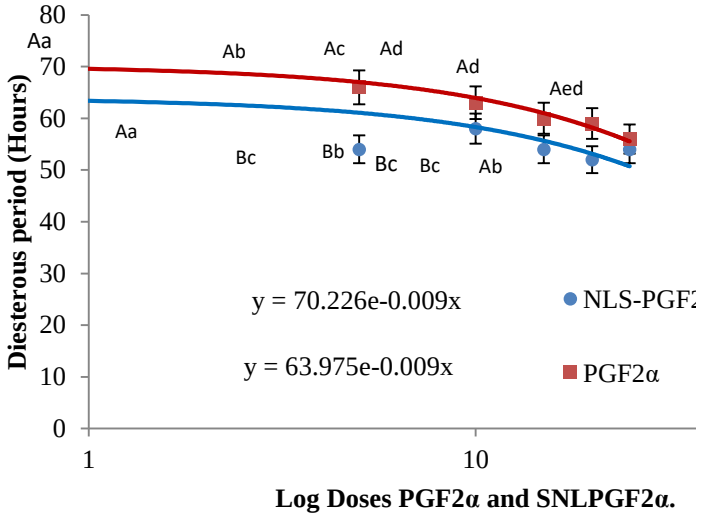


Figure (3): Log dose response curve of estrous cycle period indices of phases: Total estrous, pro-estrous, Met-estrous and diestrous, post synchronization and superovulation Protocol for PGF2, including both standard and SNL pharmaceuticals. Statistically significant (p0.05) differences between dosed forms of conventional and NLS hormones (n = 10 mice; tiny letters signified variations within dosed groups).

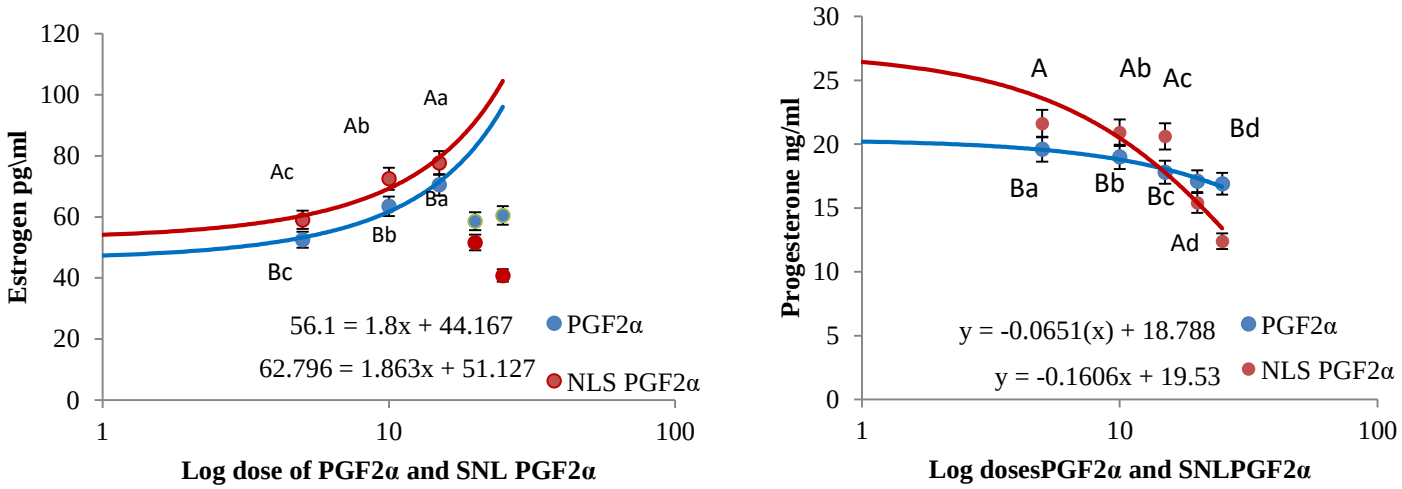


Figure (4): Hormone (Progesterone and Estrogen) concentrations (in both traditional and SNL pharmaceutics) shown against log dosage in response to the Sync-SupO PGF2 regimen. Statistically significant (p0.05) differences between dosed forms of conventional and NLS hormones (n = 10 mice; tiny letters signified variations within dosed groups).

DISCUSSION

The results showed that the highest degree of synchronization was obtained with the nano-treatment group in high doses, as the time was short and the degree of synchronization was high for estrus. direct effect of PGF2α which managed the Estrus cycle onset through Corpus leutum lysis and by promotion and evoked follicular phase then subsequent ovulation. prostaglandin was worked at molecular bases. PGF2α activates the phospholipase C pathway the provoked cellular osmo-changed [(17)], that on suggesting a mechanism of ovarian luteolysis which was noted mechanism a very well know initiation of apoptosis [(18)]. Another process was associated with non-receptor Kinase activation via stimulation Src mediated intracellular scuffled phosphorylation cascades, presumable endorse on CL regression. CL marsh bloody source of uteri ovarian and started degeneration of blood vessels so that may be derived synchronization [(19,20)].

The oxytocic impact of PgF2 on the corpus leutum may play a role in the occurrence of luteolysis through a neuroendocrine mechanism, as its initial catalytic decrease likely supported down-regulation of P4 receptor that leads to rebound retention of oestrogen activity centrally, which in turn prompted the frequency modification of the hypothalamic oxytocic puls. For example, the Estrogenic impact may raise and evoke the luteolytic puls production of endogenous uterine PGF2 secretion, a significant event of recollections of CL and uterine estrous cycle cycle synchronisation, which magnified the transduced oxytocin (21).

First, there was the function degradation stage, which was marked by a decline in progesterone levels that was consistent with the experimental results, and second, there was the exert apoptosis step, which may be thought of as the programmed death of luteolysis cells. Cholesterol transport was blocked by PGF2 (22). Dismissing the molecular regulation of angiogenic in the corpus leutum via the PgF2 -Oxytocin pathway may lead to CL dissolution and leuteal damage. This is because the PgF2 -Oxytocin pathway is associated with basic fibroblast growth factor 2 (bFGF) and angiopoietin, both of which are endocrine factors that are involved in the step-by-step regeneration of CL (23).

Changes in dosing from PGF2 (24) to Nano-form have been shown to increase both the efficiency and duration of action by virtue of the Nano-size-dependent, form's increased and intensified activity structure Nano-Lipid (25,26). This may have occurred because PGF2 re-concentrates in the utero-ovarian artery counter-current vein, thereby altering the effect of luteolysis and adjusting ovarian luteal regression. as learning to control hormones locally before they reach the ovaries (27).

CONCLUSION

The present study confirmed the high-efficiency f Nano-lipid of Prostaglandin F2α for the Synchronization of estrus cycle.

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Author Contribution: a comparable amount of work was done by each of the writers..

Conflict of Interest: The author said that there were no potential conflicts of interest..

REFERENCE

1. **Guijas C, Montenegro J, Warth B, Spilker M, Siuzdak G (2018):** Metabolomics activity screening for identifying metabolites that modulate phenotype. Nature Biotechnology, 36(4):316-320.
2. **Auwera I, Hooghe T (2001):** Superovulation of female mice delays embryonic and fetal development. Hum Reprod., 16:1237-1243.
3. **Gowda G, Zhang S, Gu H, Asiago V, Shanaiah N, Raftery D (2008):** Metabolomics-based methods for early disease diagnostics. Expert Review of Molecular Diagnostics, 8(5):617-633.
4. **Oliver S, Winson M, Kell D, Baganz F (2018):** Systematic functional analysis of the yeast genome. Trends in Biotechnology, 16(9):373-378.
5. **Carlos S, Carlos T, Geula G (2007):** The Molecular Control of Corpus Luteum Formation, Function, and Regression. Endocrine Reviews, 28(1):117-149.
6. **Pallares P, Gonzalez A (2009):** A new method for induction and synchronization of oestrus and fertile ovulations in mice by using exogenous hormones. Laboratory Animals Journal, 43:295-299.
7. **Channing C (1980):** Ovarian follicular and luteal physiology, in International Review of Physiology. Park Press Baltimore, 6:117.
8. **Iqbal M, Md S, Sahni J, Baboota S, Dang S, Ali J (2012):** Nanostructured lipid carrier's system: Recent advances in drug delivery. J Drug Target., 20:813-830.
9. **Ali N, Al-Juthery H (2017):**The application of Nanotechnology for micronutrient in agriculture production (Review Article), Iraqi Journal of Agricultural Sciences, 48(4):489 -990
10. **Kovacevic A, Savic S, Vuleta G *et al.* (2015):** Polyhydroxy surfactants for the formulation of lipid nanoparticles (SLN and NLC): Effects on size, physical stability and particle matrix structure. International Journal of Pharmaceutics, 406(2):163-172.
11. **Yang W, Cheng Y, Xu T, Wang X, Wen L (2016):** Targeting cancer cells with biotin dendrimer conjugates. European Journal of Medicinal Chemistry, 44(2):862-868.
12. **Sperelakis N (2011):** Cell physiology, Source Book Essentials of membrain biophysics. Academic Press, https://books.google.com/books/about/Cell_Physiology_Source_Book.html?...
13. **Venkateswarlu V, Manjunath K (2004):** Preparation, characterization and in vitro release kinetics of clozapine solid lipid nanoparticles. J Control Rel., 95:627-638.
14. **Mohaned A, Khamas W (2015):** The important of implement and enforce of standardize guideline for the care

and use of laboratory animals in research and teaching in Iraqi scientific institution. TJMS., 2(1):36-43.

15. **Pallares P, Gonzalez A (2009):** A new method for induction and synchronization of oestrus and fertile ovulations in mice by using exogenous hormones. Laboratory Animal, 43:295-299.
16. **Meclean A, Niclson V, Stephen F, Steffany A (2012):** Performing vaginal lavage crystal violet staining and vaginal cytological identification. Jvis Exp., 67:4389.
17. **Chen X, Xingji Y, Weina L, Qianqian S (2015):** Prostaglandin F2α regulates the expression of uterine activation proteins via multiple signalling pathways. Society for Reproduction and Fertility, 149:139-146.
18. **Aboelenain M, Kawahara M, Balboula A *et al.* (2015):** Status of autophagy, lysosome activity and apoptosis during corpus luteum regression in cattle. J Reprod., 61:229-236.
19. **Abdulkareem A, Muhammad S (2021):** Effect of Kispptin -10 As Analternative to ECG in Estrus Synchronization Protocol on improving the reproductive performance Karadi Ewes. Iraqi Journal of Agricultural Sciences, 52(3):535-546.
20. **Ogbechi J, Hall B, Sbarrato T *et al.* (2018**): Inhibition of Sec61-dependent translocation by mycolactone uncouples the integrated stress response from ER stress, driving cytotoxicity via translational activation of ATF4. Cell Death Dis., 9:397
21. **Randel R, Lammoglia M, Lewis A, Neuendorff D, Guthrie M (1996):** Exogenous PGF2α enhanced GnRH-induced LH release in postpartum cows. Theriogenology, 45:643-654.
22. **Todd W, Sandhoff M, Mark P (1996):** Prostaglandin F2α reduces steroidogenic acute regulatory (StAR) protein messenger ribonucleic acid expression in the rat ovary. Endocrine Reviews, 5:183-190.
23. **Carlos S, Carlos T, Geula G (2007):** The Molecular Control of Corpus Luteum Formation, Function and Regression. Endocrine Reviews, 28(1):117-149.
24. **Martins J, Policelli R, Pursley J (2011):** Luteolytic effects of cloprostenol sodium in lactating dairy cows treated with G6G/Ovsynch, Journal of Dairy Science, 94(6):55-76.
25. **Zebon S, Eesa M, Hussein B (2020):** Efficacy of Nano Composite Porous 3D Scaffold of Crab Shell and Al-Kharit Histological and Radiological for Bone Repair in Vivo. The Iraqi Journal of Veterinary Medicine, 44(2):15-24.
26. **Mustafa A, Khalisa K (2018):** Role of Salvia officinal's Silver Nanoparticles in Attenuating Renal Damage in Rats Exposed to Methotrexate (Part I). The Iraqi Journal of Veterinary Medicine, 42(2):7-20.
27. **Mccracken J (1971):** Prostaglandin F2a and corpus luteum regression. Ann NY Acad Sci., 180:456-472.