MUCO-ADHESIVE GEL OF LIPOSOMAL PROGESTERONE AND LIPOSOMAL PMSGVAGINAL FORMULA CHARACTERIZATION AND PREPARATION *IN VITRO* AND *IN SITU* INVAGINAL MUCOUS OF EWE

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ABSTRACT

Medroxyprogesterone acetate bearing Nano-liposomes in mucoadhesive geltablet (MAG Lipo P4)and Liposomal PMSG (Lipo PMSG)were prepared by the thin film method and evaluatedfor severalstandardization techniques and compared with Medroxyprogesteroneacetate sponges (MAPS) implicated the mucous of ewe attributes. The progestationalbioactivity of liposomal form and MAPS as compared with control was assessed by monitoring the effect on the formation of synchronization and superovulation;the liposomes encapsulate 75.41% of MAG Lipo P4 and 69.06% for Lipo PMSG. The liposomal progesterone was incorporated intoCarbopol-HPMCmucoadhesivegel and the hormone release in the vaginal strip was estimated and withdraws of degradable tablet determined *in vitro*~6. The Lamellarof the liposome in MAG Lipo P4 1-3 and Lipo PMSG 2-6 lamellae, Size of the liposome in MAG Lipo P492.19nm andLipo PMSG63.10nm, the osmotoleranceapproved set tolerated between 0.6-1% of NaCl concentration and pH challenge was stable between 6 to 8 of pH of the liposomal releasing hormone. The color of mucous share MAG Lipo P4 treated ewes were cloudier to less clear milkieras compared to MAPS treated ewes cloudyThe spinnbarkeit mucus score showed in MAG Lipo P4 treated ewe increase as compared with control groups and MAPS. The estrous cycle synchronization challenge showed Sodium, Potassium, Calcium, and

Magnesium in vaginal mucus of MAG Lipo P4 were higher than other groups, Chloride and Magnesium were similar in MAPS and MAG Lipo P4 the Potassium in MAPS as higher than MAG Lipo P4 and both was lower than both controls groups. The protein concentration of mucus in MAG Lipo P4 ewes markedly increase as compared with MAPS treated ewes and both higher than control groups

Mucus pH in both MAG Lipo P4 and MAPS was less acidic; nearby neutral, than positive control and negative controls.

It can be concluded that MAG Lipo P4 formulation tablet containing progesterone exhibited potentialeffect by *in vitro*progestational alteration of mucous content minerals and proteins and positive effect on spinnbarkeit. Moreover, an orchestrate regulation ofthe hormonal releaseand duration was achieved through the formation ofthe depot in vaginal mucosawith conducted minimalized dose and achieved better effects. However, detailed formulation use with promotion anew clinical dosage form less adverse reaction to establish the utility of synchronization and superovulation.

INTRODUCTION

The properties of the vagina cavity, dense blood vascularized system with current count phenomena shared a blood supply; organized and controlled trans-mucosal delivery of both local diffused agents and systemicdistributing therapeutically intensify active drugs (1 and 2). Drugs delivered via the vagina are not subjected to the first-pass effect and gastrointestinal interferences with the absorption of the drug are avoided that established by the greater bioavailability of hormones. Vaginal administration regularly minimizes side or adverse effects associated with the oral route (3).Mucoadhesive drug delivery system can be used to deliver the drugs for a long time and improving the bioavailability of hormones (4). Mucoadhesive formula has to prolong the interaction and increase persistence time of delivery system at the mucous membrane, because of the extended residence and local drug efficiency and/or systemic remedy uptake is encouraged and improved (5) As a progressive miniaturization formula then hydration approach to bio-adhesive delivery agent, the dry drug absorbs moisture and convert to become a gel and release active ingredient in a "time-controlled manner'. The newly developed strategies have been followed to improve the delivery of drugs through the

vagina, and among these is the use of Nano-particulate carriers based on lipids. The liposome can be used as a vehicle for administration of incorporated drugs and can provide controlled release of the drug. The Nano-Liposomes have simulated an artificial cell membrane created from cholesterol and phospholipids, its designed tensioner of concentric lipid bilayers "enclosing discrete aqueous spaces"(6). The advances in drug delivery systems for targeted delivery have been developed (7), the documents proven liposomes increase facilitation of the solubility of the drug entrapped and improve their pharmacokinetic profiles referred to the rapid metabolism, attenuated adverse effects, an increase of drug potency (8). The liposome delivery system was approved by the FDA lately (9and 10).

Vaginal mucus a complex biological fluid, hydrogel layer, and non-Newtonian forms, play forceful barrier selectively to the approval pass of particulates and microorganisms invasion (11).The endogenous hormone is regulated and controlled estrous cycle and phases consequences, and in cases of synchronized or super-ovulated ewes, as well as exogenous hormones are likewise at key role of changes of mucus. In the ewe, maximized production of clear, watery mucus is indicative of estrus onset (12) and exhibited the trending to be more basic; pH ≥7.5, through the dominance of progesterone in the luteal phase (13).

Medroxyprogesterone Acetate, Since the 1960s, the traditional method for synchronizing estrus in small ruminants through the breeding and anestrous seasons has been through the use of polyurethane sponges. Progesterone slows the frequency rate of liberation of gonadotropin-releasing hormone (GnRH) from the hypothalamus and rounded the pre-ovulatory LH surge. Apparent half-life: 30 hours to 50 days and high protein binding ~ 90% (14 and 15).

PMSG shortly, the description of gonadotropins dynamic and secretion was presented by Zondek double chain of gonadotropins (prolan A and B) in the blood and urine. Now know there are two hormones first as follicle-stimulating hormone (FSH); half-lives range 3 to 4 hours, lower clearance rates translate into increased bioactivity().

Lately, studier investigation has been attentive on vaginal drug delivery systems as logical substitution or alternatives to oral or partially parenteral hormonal and other drug administration (16 and 17). There are several dosage forms past and current studies

aimed to the advance to develop vaginal drug delivery systems for hormone and several innovative dosage form preparations for vaginal delivery systems may lead to; prolonged product shelf life, reduce dose amount, minimize toxicity, adverse effect, increase efficiency, increase stability, and improve pharmacokinetic properties(18 and 19) Tablets are the excellent bio-adhesive drug form (20, 21 and 22). The aim of the study was todesign a preparation for vaginalmucoadhesive gel liposome carrying progesterone tablet and liposomal PMSG and asses the tablet dosage form *in vitro* and *in situ* mucous changes

MATERIALS AND METHODS

The experimental protocol was conducted at the College of Veterinary Medicine-University of Baghdad/ Department of Surgery and Obstetrics. The laboratory of preparation of MAG Lipo P4 and Lipo PMSG formulations were done in Department of Physiology Biochemistry and Pharmacology, The experiment was accomplished in Station-privet field, located in Baghdad/ Abo-Graib (Al-Asass) and Al-Sayafia (Al-Sabrine village), .The ethical program followed by report Al-Bayati and Khamas (23) "standardized guidelines for the care and use of laboratory animals in research" of Iraq institutes. The experiment strategy was planned as a double-blind placebo equal design.

MAG Lipo P4 and Lipo PMSG formulations

The liposome formation process was achieved by several steps described by (7 and 24). Liposome was prepared according to the Bangham thin film method, using a technique as following Phosphatidylcholine 0.25g and cholesterol 0.25g were mixed in 1:1 (w/w) after being dissolved in chloroform 5 ml and methanol 2.5 mix combinations 2:1 v/v and vortexed for 30 min, then the solvents were evaporated by reducing pressure with the vortex. The dry film was achieved pro-liposome, the formation of Empty liposomal and entrapped phosphate buffer(25 and 26).

Liposome-entrapped progesterone

Pro-liposome was hydrated with phosphate buffer;pH 4.5 (86), containing Medroxyprogesterone acetate 150 mg. Liposomes were entrapped progesterone and formed after 30 min vortex mixing formation of liposomal progesterone (27).

Liposome-entrapped PMSG

Pro-liposome was hydrated with phosphate buffer containing Pregnant Mare Serum Gonadotrophin (PMSG) 1000 IU. Liposomes were entrapped PMSG and formed after 30 min vortex mixing formation of liposome encapsulated PMSG (28).

Muco-adhesive gel and liposomal incorporation

Carbopol 0.125 g was dispersed in distilled water 6.25 w/w 2% by stirring at 800 rpm for 60 min and adjusted with NaOH 10% dropwise was added. The substance was mixed until a transparent gel formed and the gel pH was adjusted to 4.5 (7). HPMC 0.125 g was dispersed in distilled water 6.25, w/w 2% by stirring at 500 rpm for 60 min. The gels were reserved at 4°C 12 h before using toremove air bubbles (29). The liposome encapsulated progesterone and empty liposomes were mixed into the gels individually. The liposome amount in the hydrogel was 50% 1:2 (w/w), liposome/total gel (30 and 21) fig 1.

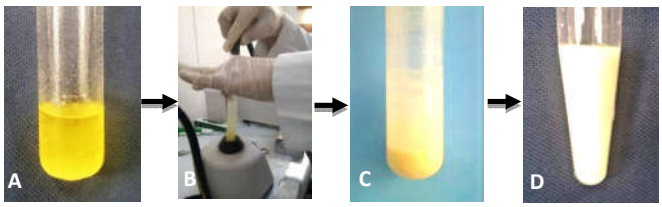


Figure 1: The process of MAGLipo P4 formulation; A: solution of chloroform-methanol 2:1 mixed with phosphatidylcholine and cholesterol in 1:1, B: vortex and evaporation liposome formation then P4 or PMSG mixed, C: entrapped hormone, D: Carbopol®,and HPMC K100M mucoadhesive gel formation.

Determination size and lamellar of liposome

light microscopic:

Checkup of both liposomes encapsulated P4 and liposome encapsulated PMSG by light microscope for describing the particle size, type, and Lamellar. The prepared liposome; 50µl of 1% liposome suspension was smeared and inspected by oil immersion (32 and 33).

Electron microscope (scan and transmission)

The preserved liposome; 0.5 g, −20°C were packed in a sealed glass container of both P4 and PMSG. The micrographs were done in Abcam, USA, for E.M. scan and transmission image aimed at lamellar and sizing of the liposome by laser beam scatting technique (34 and 35).

Determination of entrapment of liposome

The prepared liposome was centrifuged at 5000 rpm for 15 min; 4°C separate the free hormone non-entrapped for each. The hormonal amount of entrapment liposomes were extracted from the liposomal pelt formed was dissolved in methanol 1 ml and re-centrifuged, the supernatant yield was checked by radioimmunoassay as the real entrapped amount, and the entrapment was calculated according to Jassim and Al-Bayati (36) and Muneer*et al*. (37)

$$Entrapment\ \% = \frac{Amount\ of\ hormone\ in\ the\ sediment}{total\ amount\ of\ hormone} \times 100 \qquad EE\ \%$$

$$= \frac{total\ amount\ of\ drug - free\ drug\ amount}{total\ amount\ of\ drug} \times 100$$

MAG Lipo P4 formula shape:

The final shape of MAGLipo P4 was set in plastic casting set; 0.5×0.1 cm, the last compound materials were weighted and melt on whole and pressed 2 bars

Osmotolerance of MAG Lipo P4 and Lipo PMSG

Osmotic stress-induced liposomes to tolerate was measured concentrations of progesterone at zero time and after one hour as an indicator of the degree of liposomes

rapture 0.1 g MAG Lipo P4 or PMSG 1.5% were incubated with various osmolality's; hypo-hyper tonic saline solution NaCl 0.0 to 2 % involved isosmotic solution at 25 °C to challenge the vesicles. The de-encapsulated-lysis liposome liberates progesterone was separated by centrifugation at 3000 rpm for 5 min. The released progesterone in the supernatant was measured by Radio Immuno Assay and scaled with liposome number (38and 39).

1.1. MAG Lipo P4 and PMSG in pH tolerance

The stability of MAG Lipo P4 was checked by determining the pH changes on time-dependent leakage of progesterone from liposomes with different pH values prepared in normal saline pH (7.4) modified pH by HCl and NaOH for achieved pH 2, 3, 4, 5, 6, 7, 8, 9. The influence of liposome liberated progesterone concentration at zero time and after 1 hour as an indicator of liposome stability of liposomes lost structure via incubated 0.1 g MAG Lipo P4 or Lipo PMSG 1.5; acidic to the alkaline solution at 25 ºC. The de-capsulated liposome liberates progesterone was separated by centrifugation 3000 rpm for 5 min, The free progesterone in the supernatant was measured by Radio Immuno Assay and scaled with liposome number (40).

Determination of mucoadhesion time and releasing time *in vitro*

The interval of mucoadhesion of the gel formula of P4 release into the vaginal ewe mucosa was estimated according to the method of Khan *et al*. (2010) modified partially. A MAG Lipo P4; one pill, added in a water bath at 37 °C and then put, by applying with the fingertip a light pressure for 30 s, on a section of ewe vaginal mucosal strip. This set was placed at an angle of 20° in a chamber at 34 °C and exposed to a pH 6.3 simulated vaginal fluid: 8.77 NaCl, 2.98 KCl and 0.59 $CaCl_2$mg/ml, with shaking every day; showed in fig 2. The time required for completing the washing of the formulation, noticed on the basis of the cumulative P4 release, was measured as the mucoadhesion time and releasing percent *in vitro* (30 and 41).

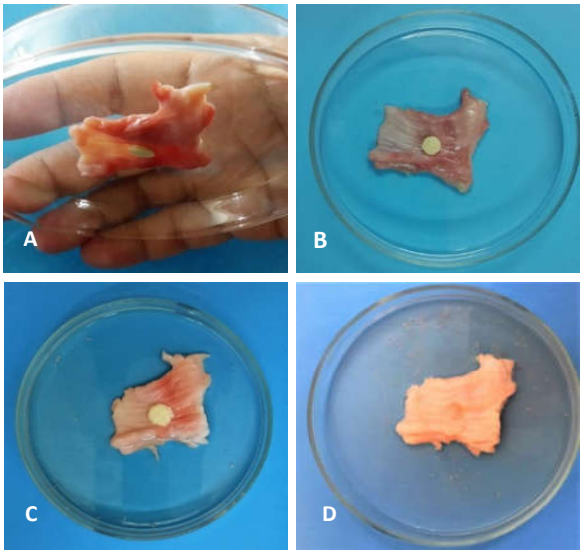


Figure 2: release and adhesive of MAG Lipo P4 technique on vaginal strip rinsed in mucus stimulant media *in vitro*. A: upside down of MAG Lipo P4 on the vaginal strip, B: adhesive MAG Lipo P4 at zero time of incubation, C: MAG Lipo P4 adhesive at the third day of incubation, D: showed the degradable detached MAG Lipo P4 at the seventh day of incubation.

Animal management

Forty ewes were group-housed in the straw-bedded arena. Ewes' age were ranged between 2 – 4 years old, and a body weight of 45±2.7 kg. None of the ewes used in these trials had been previously subjected to any program (42).

Experimental design

A fully crossed design (Factorial design), Ewes were distributed randomly into four groups; 10 ewes in each

1st: negative control, 2nd: Positive control with a MAGLiposome Empty intra-vaginally application, 3rd: Medroxyprogesterone acetate sponges; MAPS (20mg) intra-vaginally application and PMSG (200 IU) IM, 4th: MAG Lipo P4 5 mg and Lipo PMSG 50 IU Intra-vaginally adhesion.

Collection of vaginal mucus

Vaginal mucus was collected from the vaginal zone through aspiration using an adapted pipette joined to a 10 ml syringe. The sterile pipettes were spraying normal saline for moisten insertion. The collection of mucous initiated a day prior to the

estrus onset and continued for 12 days in total. Collections occurred at 24-hour intervals; (8 am) for 3 days, the collected mucus was transferred into conical tubes and centrifuged; 1500 rpm for 15 min., to precipitate cellular debris with the supernatant reserved (43).

Color of Mucus

The color was evaluated via a scoring system from 1-7, based on observations table 1 of color marks-Score table 1 (13)

Table 1: The score of mucus color markers (44)

Scores	Remarks
1	Clear
2	clear-cloudy
3	Cloudy
4	cloudy-milky
5	Milky
6	milky-creamy
7	Creamy

Mucus spinnbarkeit

The mucus spinnbarkeit was scored when mucous was recovered; the Spinnbarkeit measured by a 50 µl of mucus was sited between two coverslips closed moved vernal scale the coverslip was gradually detached, till the mucous strand between them was broken. The extend distance of mucous strand was assessed in centimeters and assumed a score showed in table 2(44)and fig. 3

Table 2: The score of spinnbarkeit indicate to a distance of mucus extent

Scores	Distances cm
1	< 0.1
2	> 0.1
3	> 0.5
4	> 1.0
5	> 1.5
6	> 2.0
7	> 2.5

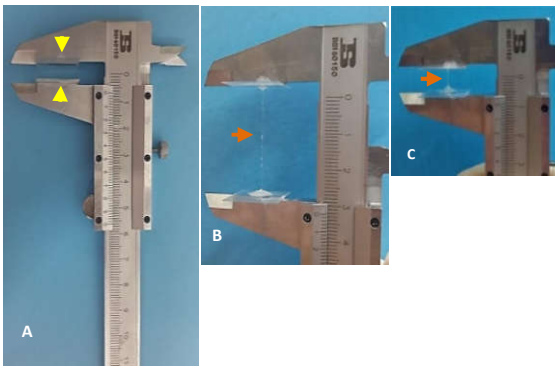


Figure3: Spinnbarkeit modified tool; A verinea was modified and close attached coverslip (yellow arrow), B and C spinnbarkeit tool hold mucus and spread for thread strand (pink arrow) measurement.

Chemical assessments of mucous

Aliquot of Mucous 0.5 ml was assessed for the chemical profile (mmol/L); Sodium, Calcium, Potassium, Chloride, and Magnesium. Specimens were evaluated by the following: Chloride, Magnesium, and Calcium were measured using Mercury/Iron thiocyanate, CPZ, and Arsenazo III respectively, and estimated by spectroscopy techniques. Both Potassium and Sodium were assessed via flame photometry techniques (45).

The Protein concentration of mucus

The protein concentration (µg/µl) of mucous Specimens pools was determined using a Pierce bicinchoninic acid assay (46).

Determination pH of mucous

The pH of samples was determined in vivo using a paper strips type with measurements taken from the vaginal mucus pool just prior to sample collection (13).

Statistic

F test was used analysis data by analysis of variance (ANOVA) ANOVA two way analyses and comparison

RESULTS

Liposome size

The results of liposomal size were shown in table 3 the MAG Lipo P4 and Lipo PMSG size were 92.19 ± 4.06 nm (54.82 - 148.51) and 63.10 ± 5.21 nm (38.38 - 94.22) respectively. The MAG Lipo P4 was significance ($p<0.05$) larger than Lipo PMSG.

Table 3: Size of the liposome in MAG Lipo P4 and Lipo PMSG

Liposomal hormone	Size of liposome nm	
	Range	Mean ± SE
MAG Lipo P4	54.82 - 148.51	92.19 ± 4.06 *
Lipo PMSG	38.38 - 94.22	63.10 ± 5.21 **

The data presented as mean ± SE, The stars denoted ($p<0.05$), n: 10, MAG Lipo P4: Muco-adhesive gel of liposomized progesterone, Lipo PMSG: liposomal pregnant mare serum gonadotropin

Liposome lamellar

The table 4 exhibit the lamellar appearance of the liposomal shell, The lamellar was ranged 2-6 (3.26 ± 0.13) of MAG Lipo P4 as more as Lipo PMSG 1-3 (1.42 ± 0.28) significantly ($p<0.05$).

Table 4: Lamellar of the liposome in MAG Lipo P4 and Lipo PMSG

Liposomal hormone	Lamella number of liposome	
	Rang	Mean ± SE
MAG Lipo P4	2 - 6	3.26 ± 0.13 *
Lipo PMSG	1 - 3	1.42 ± 0.28 **

The data presented as mean ± SE, The stars denoted ($p<0.05$), n: 10, MAG Lipo P4: Muco-adhesive gel of liposomized progesterone, Lipo PMSG: liposomal pregnant mare serum gonadotropin

The depiction 4of MAG Lipo P4 showed the layers of liposomal shell in light microscope multi-vesicles liposomes and electron micrograph transmission manifested multi-lamellar feature figure (5) with the obvious basic central hole of the liposome. The scan EM depiction showed the globular and cavities of the location of the liposome in the general formula.

The Lipo PMSG formula showed in light microscope depiction vesicle liposome harmony and uniformity of shape appearance and as well as the general size. The electron micrograph showed the uni-lamella and bi and triple-lamellar with a large central whole, the feature described as small uni and bi-lamellar.

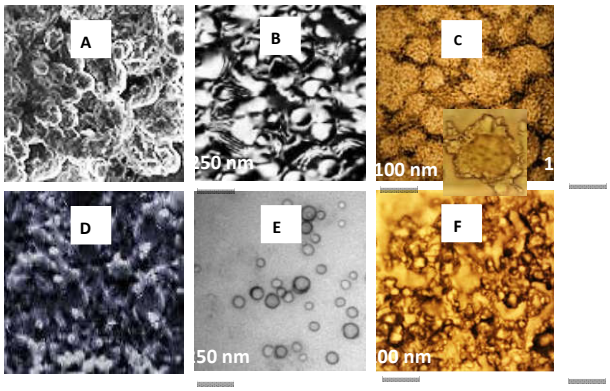


Figure 4: Liposome micrograph A, B, and C MAC Lipo P4 and D, E and F Lipo PMSG; A scan microscope depiction liposome look like globular appearance. B transmission electron micrograph of liposome was indicative multi-lamellar. C light microscope designed as grape-like appearance indicative to multi-vehicle. D electron microscope scan depiction liposome was looked like small vesicles harmony size. E electron microscope transmission depiction appeared small uni and bi-lamellar. Flight microscope depiction showed small liposome. MAG Lipo P4: Muco-adhesive gel of liposomized progesterone, Lipo PMSG: liposomal pregnant mare serum gonadotropin

Liposome entrapment

The hormonal entrapment in table 5 showed entrapment percent of MAG Lipo P4 75.41 ± 3.95 and entrapment efficiency % was 81.39 ± 7.02, whereas, Lipo PMSG entrapment percent was 69.06 ± 5.14 and Entrapment efficiency was 87.55 ± 4.56.

Table 5: Liposome entrapment of hormone in MAG Lipo P4 and Lipo PMSG

Liposomal formulas	EA	NEA	EE%	E%
MAG Lipo P4	113.11 mg	27.92 mg	81.39 ± 7.02	75.41± 3.95
Lipo PMSG	690.60 IU	86.55 IU	87.55 ± 4.56	69.06± 5.14

The data presented as mean ± SE, The stars denoted ($p<0.05$), n: 10, MAG Lipo P4: Muco-adhesive gel of liposomized progesterone, Lipo PMSG: liposomal pregnant mare serum gonadotropin, EA: Entrapped amount, NEA: Non- entrapped amount, EE: Entrapment efficiency, E: Entrapment

MAG Lipo P4 formula shape

The MAG Lipo P4 shape categories were discoid and flatten harsh side and smooth side, diameter 0.5 cm radius and 0.1 cm thickness and color white-pale hard gel (fig 5).

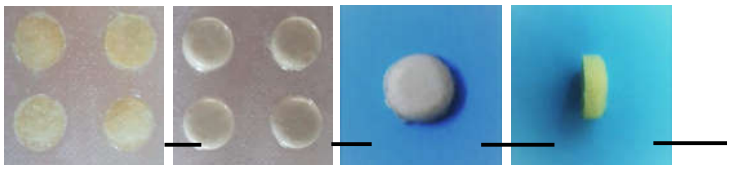
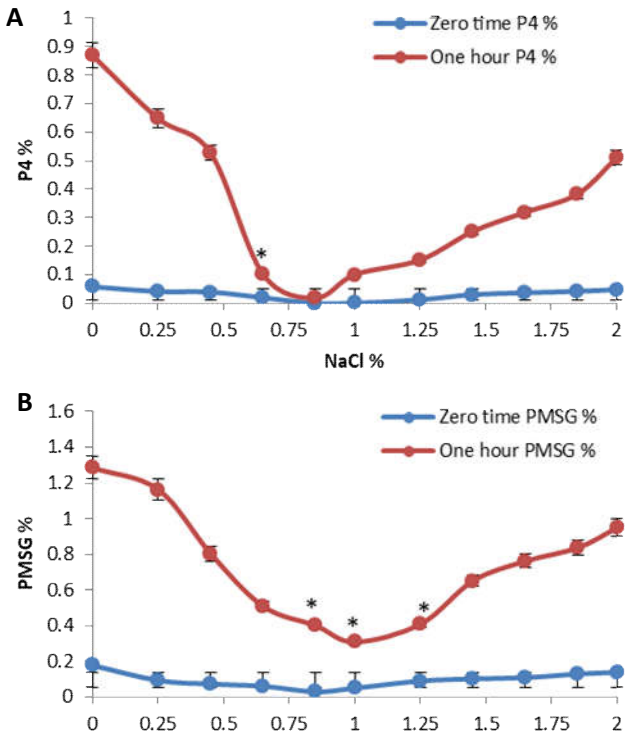


Figure 5: MAG Lipo P4 presented form; Bars denoted 0.5 cm

Osmotolerance of MAG Lipo P4 and Lipo PMSG *in vitro*

The osmosis tolerance in figure 6 exhibited the relationship between hormonal release from liposomal formulas and NaCl tonicity at both hypotonic to a hypertonic solution. The hormonal released or concentration was increased significantly ($p<0.05$) run parallel with hypertonic and hypotonic solution correlated negatively of MAG Lipo P4 and Lipo PMSG. The hormonal concentration in different NaCl concentrations after one-hour incubation superior to that of zero time in both hormonal formulas



The data presented as mean ± SE, n= 5, MAG Lipo P4: stars denoted P<0.05, Muco-adhesive gel of liposomized progesterone, Lipo PMSG: liposomal pregnant mare serum gonadotropin

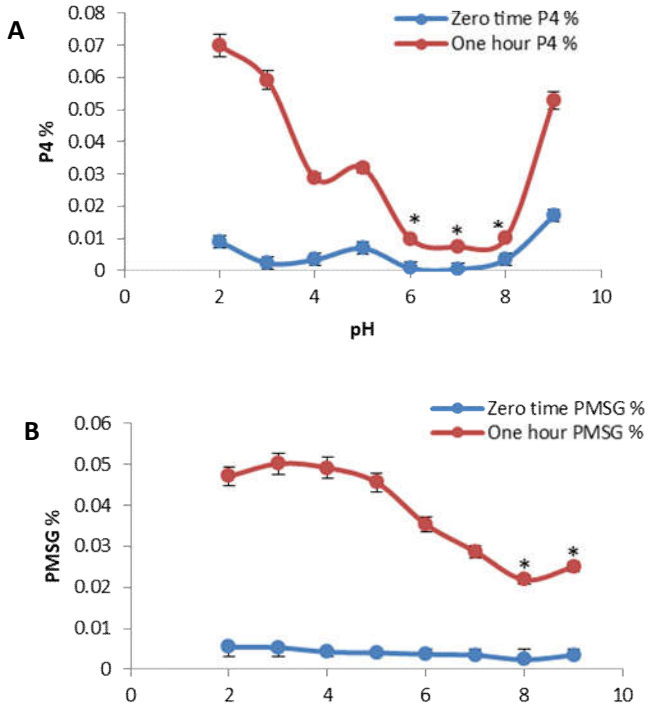
Figure 6: The effect of turbulence osmosis changes on the MAG Lipo P4 and Lipo PMSG tolerance in a serial solution of hypotonic and hypertonic media *in vitro*

The pH tolerance of MAG Lipo P4 and Lipo PMSG

The depiction (6A) displayed significant (p<0.05) P4 increase release and reduced tolerance of MAG lipo P4 associated with a reduction of pH values (acidic phase) and less in basic medium whereas, the in neutral pH showed not remarkable turbulences with stable flush releasing and less than other pH phases. The one hour evolution time showed significant higher than zero time in all pH medium.

The figure (6B) of Lipo PMSG - pH tolerance relationship displayed significance (p<0.05) increased PMSG release associated with decrease pH values and indicated consequence dispossess of liposomal tolerance in acidic phases.

The one-hour exposure of Lipo PMSG to pH media values showed significant (p<0.05) as compared with zero time exposure



The data presented as mean ± SE, n= 5, pH stars denoted P<0.05, MAG Lipo P4: Muco-adhesive gel of liposomized progesterone, Lipo PMSG: liposomal pregnant mare serum gonadotropin

Figure 6: The effect of pH on the MAG Lipo P4 (A) and Lipo PMSG (B) tolerance in alkaline and acidic media in vitro

Determination of mucoadhesion MAG Lipo P4 time and time of the release of P4

Figure 7 presented the releasing of P4 of MAG Lipo P4 on vaginal strips in muco-stimulant solution with determining the tolerance of adhesion per days, The lag time of release was 80±9.15 minutes and the first three days 1-3 day showed slow release of P4 appears bulge with granulation of gel pill, and the three consecutive days 3-6 days fast releasing of P4 and pill swelling and fragmentation and still adherent, and the final three days 3-9 day of releasing and adherent showed disintegration of gel pill with cessation of released P4.

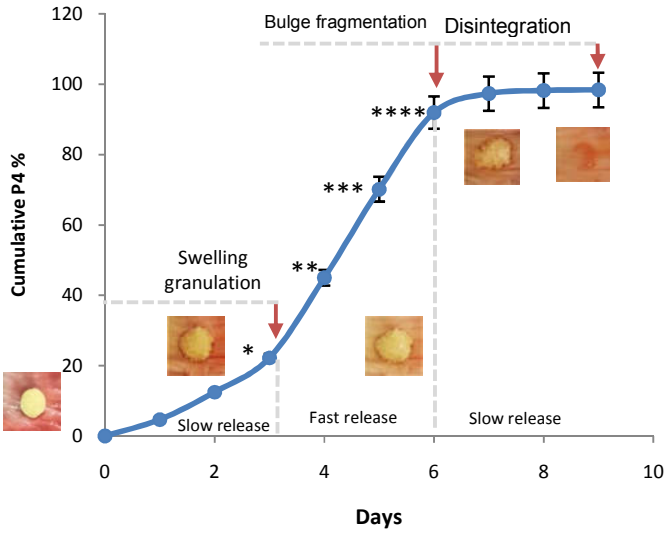


Figure 7: the release of P4 of muco-adhesion MAG Lipo P4 and time of adherence of gel pill. n= 5, Data presented mean ± SE

The data presented as mean ± SE, n= 5, stars denoted P<0.05, MAG Lipo P4: Muco-adhesive gel of liposomized progesterone.

Color of Mucus

The color of mucus in the day 10 of treatment withdrawal of MAG Lipo P4 treated ewes were cloudier to less clear milkier 3.27 ± 0.18 significantly (p<0.05) as compared to MAPS treated ewes cloudy 3.05 ± 0.22 and Positive and negative control Clear-cloudy 2.59 ± 0.30 and 2.17 ± 0.14 respectively, however, the positive control was cloudier than negative control ewe mucus (fig 8)

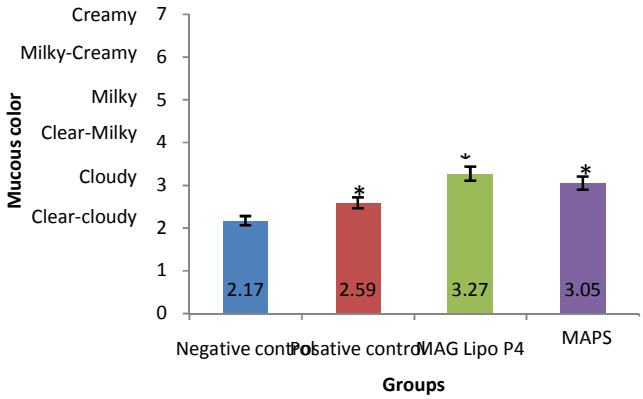


Figure 8: the mucus color of ewe treated in MAG Lipo P4, MAPS and positive and negative control

The data presented as mean ± SE, The stars denoted (p<0.05), n: 10, MAG Lipo P4: Mucoadhesive gel of liposomized Progesterone, MAPS: Medroxyprogesterone acetate sponges, - control: negative control, + control: positive control

Mucus spinnbarkeit

The spinnbarkeit mucus a score showed in figure 9, the MAG Lipo P4 treated ewe group displayed significant (p<0.05) increase as compared with other treated groups and MPAS higher than both control groups negative control and positive control, and the positive control ewes significantly score more than negative significantly (p<0.05).

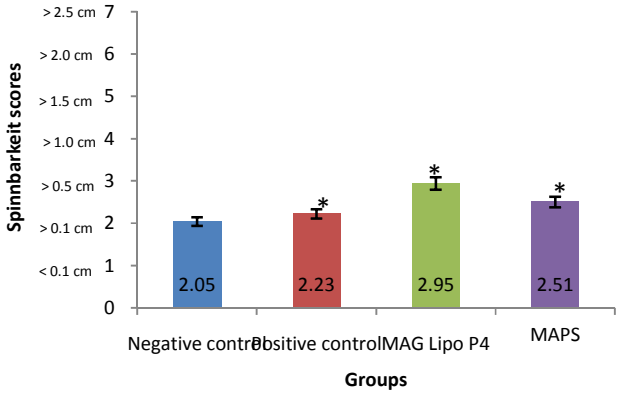


Figure 9: The mean values of spinnbarkeit score of ewe mucus synchronized estrous by MAG Lipo P4, MAPS and positive and negative control

The data presented as mean ± SE, The stars denoted (p<0.05), n: 10, MAG Lipo P4: Muco-adhesive gel of liposomized Progesterone, MAPS: Medroxy-progesterone acetate sponges, - control: negative control, + control: positive control

1.2.Chemical assessments

The estrous cycle synchronization formulas had a diverse result in levels of Sodium, Potassium, Calcium, and Magnesium in vaginal mucus (Table 4.7). Levels of Sodium and Calcium in mucus of MAG Lipo P4 ewes were higher than other groups (p<0.05), whereas the Levels of Chloride and Magnesium were both similar in MAPS and MAG Lipo P4 (p>0.05), the Potassium in MAPS as higher than MAG Lipo P4 and both was

significance (p<0.05) lower than both controls groups; negative control Chloride and Magnesium positive control Chloride and Magnesium The positive control group showed significantly increased Calcium level as compared with negative control.

	- Control	+ Control	MAG Lipo P4	MAPS
Na	98.10±8.58*	100.73±6.25*	119.35±10.70**	91.27±9.30
Ca	0.61±0.05	0.69±0.01*	0.99.73±0.01***	0.89±0.02**
K	17.03±1.11**	16.88±2.14**	14.06±2.50	13.35±1.41
Cl	110.61±10.58*	118.04±12.71*	139.77±.9.26	136.38±11.03
Mg	24.30±0.03	25.21±0.05	27.15±0.02*	26.91±0.04*

Table 6: The Levels of Sodium, Calcium, Potassium, Chloride, and Magnesium in the vaginal mucus of MAG Lipo P4, MAPS synchronized ewe, positive and negative controls ewes during the withdrawal period

The data presented as mean ± SE, The stars denoted (p<0.05), n: 10, MAG Lipo P4: Muco-adhesive gel of liposomized Progesterone, MAPS: Medroxyprogesterone acetate sponges, - control: negative control, + control: positive control

The Protein concentration of mucus

The protein concentration of mucus from MAG Lipo P4 ewes markedly increase as compared with MAPS treated ewes and both higher than control groups negative control and positive control significance (p<0.05) and there was no significant (p>0.05) between controls groups (fig.10).

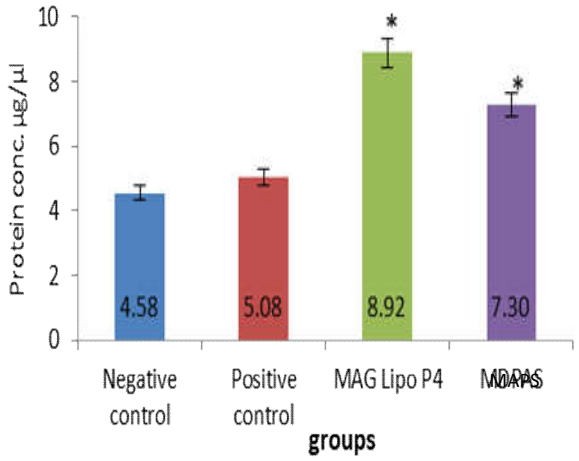


Figure 10: The protein concentration µg/µl in ewe vaginal mucus synchronized estrous by MAG Lipo P4, MAPS, and positive and negative control groups

The data presented as mean ± SE, The stars denoted (p<0.05), n: 10, MAG Lipo P4: Muco-adhesive gel of liposomized Progesterone, MAPS: Medroxyprogesterone acetate sponges, - control: negative control, + control: positive control

pH of mucous

The mucus pH values in figure 10 showed no comparable changes between MAG Lipo P4 and MAPS during withdrawal hormonal treatment formulas (p>0.05). Mucus pH in both MAG Lipo P4 and MAPS was significantly (p<0.05) less acidic; nearby neutral, than positive control and negative controls.

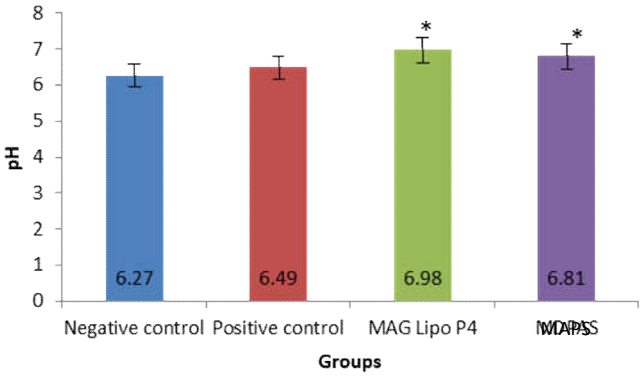


Figure 10: pH of vaginal mucus from MAG Lipo P4, MAPS, and positive and negative control groups

The data presented as mean ± SE, The stars denoted (p<0.05), n: 11, MAG Lipo P4: Muco-adhesive gel of liposomized Progesterone, MAPS: Medroxyprogesterone acetate sponges, - control: negative control, + control: positive control

DISCUSSION

The study was devoted to the improvement of an alternative sponges Progesterone-PMSG hormonal delivery and assessment of new formulation Nano-liposome for ewe synchronization and superovulation. Although the Nano-carriers described have potential applications in numerous reproductive systems, this study was dedicated to challenging an alternative sponges MAP and their short comes via mucoadhesive

liposome containing progesterone, As well as, reformulate PMSG injectable form to liposomal PMSG with a dosed minimized amount.

For this purpose, the study aimed a new Nanotechnological formulation to overcome some problems of sponge through minimized the dose, substituted the sponge by Nano-sized liposomal bio-adhesive (mucoadhesive) tablet form as well as partially reduce time of hormone exposure, and no interference of removal of formula self-withdraw degradable form (1).

The idea of alternative conventional sponge's application attributed to the mucoadhesive has advantages; it was small tablet form facilitate localized in the upper roof of vaginal tubal site of application, mucoadhesive also improved and expanded the bioavailability of applied hormone may be due to providing intimate or close contact of the formula with the mucosal layer of vagina and increased the absorption per local surface area. The mucoadhessive vaginal gel tablet small size approved with their gel content as a semisolid flexible may be believed natural texture resemble natural tissue (7).

Physical features of MAG Lipo P4 and Lipo PMSG

The multilamellar Liposome of MAG Lipo P4 and unilamellar of Lipo P4 was designed to improve entrapment of hormone and correlated with size 92.19± 4.06 and 63.10 ± 5.21 respectively table 4.4 (30). The polarity of the solvent was adetrimental factor of a prepared liposome that methanol-chloroform and methanol only for multilamellar Liposome of MAG Lipo P4 and unilamellar of Lipo P4 respectively as well as their size (47).

The hydrophobic core entrapped PMSG as glycoprotein and in both lamellar space of P4 that influences by cholesterol content and dehydration time of solvents and maintain stability via electrostatic bonds with improved capacity and giant liposomal hole and spaces (48 and 49).

The type of chemical properties of the hormone as water or lipid soluble can be entrapment determination with a degree of lipid solubility improved quantity of hormonal percentage in liposome structural module of the membrane and liposomal size (50).

Chen et al.(51) discuss the hydrophilic and lipophilic was entrapped an effective amount. The entrapping efficiency of multilamellar table 4.6 was presumably due to improving hydrating the lipid base via the preparation uses of an organic solvent (52) furthermore the entrapment efficiency of progesterone more efficient due to attributed to the lipoid nature of the drug (30). The size and the final shape of liposome loading hormone was presumably due to the positively charged protein has been localized on the surface polar head group of the phospholipids molecules, resulting an increased in the diameter of the liposomal membrane and jagged spherical surfaces (53).

Osmotic tolerance

Tolerance of osmotic changes on liposome was tested the osmotic conclusion independently on electrostatic defy, the osmotic force variability by addition of different concentrations of NaCl and has the capability to infiltrate through phospholipids bilayers. Hypoosmotic tolerance was achieved by diluting liposome different concentrations of NaCl. The hormonal liberation was checked after one hour that referred to an osmotic sporadic rapture of Liposome (54) figure 6. The hypoosmotic formed an increased bulk of particle size after one hour osmosis challenges that were showed decrease P4 under hypoosmotic condition was slightly shifted curve to the left.The explanation for this point may be due to the swelled liposome that suffers osmotic swelling that provides an impression the phosphatidylcholine - cholesterol liposomes had a fluidity and elasticity with degree compliance the increase in their size and release of P4 no escaped (55).

Chen *et al*.(51) endorsed the stability of liposomal phosphatidylcholine as an vital tolerance through the physiological osmotic affection on improved highly evaluated pharmacokinetic the maximized stability of liposomal entrapped hormone may be attributed to the improved lamellar force built, stability and an increased facility of liposome remaining in the vaginal environment per survival time.

That fact may be lead to reached C.max within longer time as well as t½ flip-flop phenomenon (56) and extend timeproduced by liposome carrying hormone than that used sponges hormone, these suggestion and attributes may be due to the liposomal hormone provided intensify effective concentration in vagina as a primary target site

then blood as firstly reflected to improved absorption that prolonged hormone steady state in plasma and therefore the increase duration of action and impacted on the endpoint of aim induction of estrous synchronization and superovulation (57).

pH tolerance

The result of pH media on liposome exhibited Inside the vaginal hormonal delivery definite targeting of the mucous attachment have exposure to variability of environmental changes valvular orifice and containment with changes of estrous phases, inaddition to stationary movement time (58). Liposome is drug carrier vehicles that can be used to keep advantageous interaction with mucous secretion through a mucoadhessive form (59).

The result showed tolerance in different pH value which reduces the number of liposome at pH indicated by increasing concentration of progesterone releasing in media and less releasing was seen between 6 to 9 pH the outcome may be presumably due to the liposomal entrapped hormone approved transit to acidic media after one hour through the electrostatic interface that govern in the liposome-entrapped hormone PMSG or P4 (60). Hydrophilic interaction is important for binding of the liposome with the hydrogen which occurs between the entrapped hormone and the phospholipid head of the lipid bilayer (61), also, Kim (62) specified another factor may be inspected as the tolerated liposome from pH changes in media may be due to the theory state that can lead to the agglomeration of lipid particles and consequently the yield of huge liposome-hormone complex as "charge-mosaic theory" (63).

Mucoadhesion MAG Lipo P4 time of P4 release

The *in vitro* vaginal permeation study revealed that the drug permeationacross the vaginal membrane from liposomal formulation follows near zero order kinetics determined using the slope of log cumulative drug permeated versus log time plot figure 4.20. The lag time and slow release of 80 ± 9.15 minutes for liposomal adhesive gel were observed. This higher lag time with liposomal formula may be due to the formation of liposomal depot in the vaginal layer wall as a consequence the permeation of drug across the vaginal membrane was retarded that resulted from a

prolonged release from swelling form of tablet, The reason of the long disintegration time of MAG Lipo P4 was the presence of disulfide bonds which, turned to be within the thiomer itself. Crosslinking of polymeric chains as a result of the development of inter and intra-molecular disulfide bonds formation in the high adhesive property due to high stability of the tightened polymeric bonds linkage and adhesive belongings. Adhesion of the delivery system over the intended period of drug liberation is also a significant requirement for achieving controlled release of the hormone. Besides, the high cohesive property of the polymeric carrier matrix is advantageous to minimize irritating vaginal outflow of eroded fragments (64 and 65). Furthermore, actually share slow release. whereas, the flowed by fast released phase due to cumulating of releasing and promote massive degradation and fragmentation and increase surface of exposure and that may be promotion of the progesterone release per time finally, the releasing was reduced and permeation cessation in disintegration phase due to exhaustion or depletion of progesterone in liposomal mucoadhesive gel tablet (30)

Mucous characterization

. Mucous color

The effect of exogenous hormones MAG Lipo P4 on the changes in muco-vaginal characteristic at the 10^{th}day of time of withdrawal showed in figure 4.21, cloud to milky appearance of mucus as compared with control groups clear to cloudy and usually changes occur during the estrous cycle, whereas, the MAPS treated group is dominance cloudy, the main factor affected on mucous color are mucus hydration and production, The clear color and low proteinaceous. The decrease in mucus production may in part be due to decreased para-cellular permeability of the ectocervical cells, which has been shown to occur after progesterone treatment in the ectocervical cell (66).

The cloudy color positive correlated with volumes resulting in a decreased ability to eliminate foreign bodies from the track and a less compatible track, the cloudy in MAPS is dominance cloudy may be due to vaginitis and their secretion caused donated color in contrast that found in MAG Lipo P4 presumably reduction of volume (67 and 68).

Moghissi and Marks (69), who presented the sheep treatment with P4, caused a lessening in the secretion of mucus and become more viscous. These results are in agreement with Smith and Allison (72), attributed the ewe treatment with exogenous P4 produced a significant decrease in the mucus secretion at estrus than untreated ewe (46).

The cloudiness color of mucus may be negatively corrected with protein content production; the protein concentration increased in MAG Lipo P4 showed in figure 4.23, and decrease mucous volume and yield viscous consistency under progesterone effect (71). In the ewe (12 and 71) they have attributed an alteration in carbohydrate and protein concentration over the cycle, with increased protein content yield in cloudier, opaque mucus (72).

. Spinnbarkeit

The results of spinnbarkeit shown in figure 6, the comparisons with some studies on mucus categories are hard due to the complex organizational and structural behavior of mucus as it is a non-Newtonian fluid,reports also vary in regards to the effect of synchronization on mucus spinnbarkeit, with progesterone synchronization subsequent decreased spinnbarkeit length in comparison to mucus of obviously cycling ewes otherwise the estrogenic effect made mucus hydrated and decrease spinnbarkeit (73 and 74). Whereas, the time of mucus collection at 10th at initiation of follicular effect dominance that attribution effect of MAG Lipo P4 and MAPS than control groups spinnbarkeit, furthermore, the Nano form MAG Lipo P4 higher than MAPS may be due to promotion P4-PMSG program more effective in spite of 4th fold lower dose of program than conventional type and this result approved that spinnbarkeit has good and sensitive indicator for estrous, this approved the results by the onset of estrous time in MAG Lipo P4 lower than MAPS.

Adams et al., (75) attributed the changes in color and spinnbarkeit to hypothetical exogenous administration of estrogen control both the quantity of mucus and the proportion of water in the mucus basically by governing the quantity of water secreted by the mucosa of the crevico-vaginal tube (46). The influence of the estrogenic effect

dominant on the proportion ratio of water content in the mucus was gotten in MAG Lipo P4 and MAPS followed this proportion.

. Chemical assessments

The results of chemical composition showed in table 6, The Ca^{++} increased in MAG Lipo P4 vaginal mucous more than other groups the Calcium (Ca^{++}) one of main ion contribution of changing the physical properties of mucous which ware Chen *et al*. (76) and Muchekehu and Quinton (77) have suggested Calcium plays a vital role in mucus structure by participation in mucin charge, bonding, release and expansion, that effects mucus swelling, hydration, structure, and viscosity (76 and 77) and as such any modification to accessible extracellular, free or mucus bound forms might modify mucus building then modified the color and spinnbarkeit.

The changes may be attributed to Per Bergman's suggestion inthe 1950s the results are accord with the optimal time of estrous behavior and fertility coincides with the "water phase of the cervical mucus; i.e., that phase of the cycle immediately preceding and coinciding with the thermal shift." The remarks on cervical mucus are practical, simple, and clinically rewarding (78). The estrogenic outcome causes hyperemia profuse exudation and hydrated mucous with increase material deposit (79 and 80). That reflected on the fertility grad of animals was directly positive correlated with the mucous amount as usual spinnbarkeit length and suggested that actually increase migration of sperm and provide the best factor improved fertility index and their belonging (81).

The levels of some minerals content of mucous showed an increase of chloride levels may be due to particular change almost parallel with the estrogen hormone. A sharp increase in chloride content typical of a marker of pre and ovulation was observed an efficient when withdrawing of P4 (82). Furthermore, the Cl^{-} ion is directly proportional to protein concentration in mucous table 7 and figure 6 and the ratio represented cervical mucus index (CMI) achieved ratio flow up spinnbarkeit and color that presumable give a suggestion the protein transport and liberation on the negative charge of cells and implicated with Ca^{++} ion mimic the mucosal cell exchanges (83).

The Na^+ concentration in the mucous increase in MAG Lipo P4 than other group that may be presumably due estrogenic effect and entered the ewe on estrous and behavioral display the treated group and control; that one of improvement the Nano-liposomal formula give an impression superior of response in Na+ and one of marker of ovulation increase of mucosal NaCl

The Magnesium increased and decreased Potassium in MAG Lipo P4 and MAPS and the tenacity of in Nano formula was higher than the sponge that suggested the ionic equilibrate the osmotic pressure of mucous implicated with other ions regulated thickness and color and spinnbarkeit.

. pH of mucus:

Earlier notions and clinical reports have documented the follicular-estrogenic phase initiation was powered the outcome in less acidic-neutral vaginal mucus (84). Nonetheless, the results in the present study showed both MAG Lipo P4 and MAPS pH closely near neutral mucus pH was higher than control groups by hormonal synchronization and superovulation. Similar work has also been attributed this changes to increase circulating estrogen levels resulting towered of basic vaginal mucus.

This discrepancy could be in part be related to presumably preceding research has found that increasing circulating oestrogen concentrations results directed the vaginal mucous to basic pH, furthermore, the ionic variation may play a role in pH variation between control set the notarized pH of hormonal treatment (48).

Possibly this variation in vaginal pH between control and hormonal application is due to alteration in usual vaginal microflora; humans and other animals having the high concentration of lactic acid formed by Lactobacillus spp, while, ruminants may have less, or perhaps a modified dominate types of microflora or by-product yield (85).

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خصائص الجل المخاطي اللاصق للبروجستيرون المحمل باللايبوسوم مصل الفرس الحامل المحمل باللايبوسوم بالشكل المهبلي وتحضيره بالزجاج وفي مخاط مهبل النعاج

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الخلاصة

حضرت استات البروجستيرون المشكل بصيغة حبه اللايبوسوم النانوي في جل المخاط اللاصق MAG Lipo P4 ومصل الفرس الحامل المحمل باللايبوسوم Lipo PMSG بطريقة الصفائح الرقيقة وقيما بتقنيات قياسيه متعددة وقورن باسفنجات المغمسة باستات البروجستيرن (MAPS) لمستوى صفات المخاط المهبلي. الفعالية البايوحيوية للهورمون اللوتيني ذو الشكل اللايبوسومي والاسفنجات المغمسة باستات البروجستيرون بمقارنتها بمجموعة السيطرة قيمت بمراقبة توحيد الشبق وافراط الاباضه. وحمل اللايبوسوم ب MAG Lipo P4 ٧٥,٤١% اما ال Lipo PMSG ٦٩.٠٦%. وحضر البروجستيرون المحمل باللايبوسوم بدمج الجل المخاطي اللاصق الكاربوبول والج بي ام سي وقيس تحرر الهورمون من القطعة مهبيله ومدة نفاذ الدواء وتفتت الحبه المدمجه باللايبوسوم المحمل بالبروجستيرون في الجل المخاطي اللاصق في الزجاج وقدرت بحوالي ٦ ايام. صفائح اللايبوسوم في MAG Lipo P4 تراوحت من ٢-٦ وحجمه ٩٢,١٩ نانوميتر وفي Lipo PMSG ٢-٦ وحجمه ٦٣,١٠، واظهرا تحملا اوزموزيا بين ٠,٦-١% من تراكيز كلوريد الصوديوم وابدى ثباتا بين ٦-٨ في قيمة الاس الهيدروجيني معبرا عنه بقيمة تحرر البروجستيرون من اللايبوسوم في الوسط. وفي مجموعة المعالجة بال MAG Lipo P4 تبين لون المخاط المهبلي غيمي الى حليبي فاتح مقارنة بمجموعة المعالجه بال MAPS بين لونه غيمي. والتقييم الرقمي للزيادة والنقصان في طول الخيط المخاطي اظهر في النعاج المعالجه ب MAG Lipo P4 زيادة بقيمته في مجموعتي السيطره والمعالجه ب MAPSان توحيد الشبق اظهر ارتفاعا بتراكيز الصوديوم والبوتاسيوم والكالسيوم والمغنيسيوم في مخاط المهبل في مجموعة MAG Lipo P4 بالمقارنه مع المجاميع الاخرى، ولم يظهر الكلوريد والمغنيسيوم فرقا بين MAG Lipo P4 وال،APSلمغنيسيوم فرقا بين ،لاخرى ووم في مخاط المهبل انوي الذي سهل امتصاص MAPS، البوتاسيوم في ال MAPS اعلى من MAG Lipo P4 وكليهما اقل من مجموعة السيطرة. تركيز البروتين في المخاط مجموعةالنعاج المعالجه ب MAG Lipo P4 ملاحظ زيادة مقارنة ب مجموعة المعالجة ب MAPS وكليهما

اعلى من مجموعة السيطرة. ان مخاط المهبل في كلا مجموعتي MAPS وMAG Lipo P4 اقل حمضه; قريب المتعادل، من مجوعتا السيطرة الموجبة والسالبة.ونجمل ان صيغة MAG Lipo P4 الحاوية على البروجستيرون تغيرات فعال في مختوى المخاط من المعادن والبروتين وتاثير ايجابي في طول الخيط المخاطي وابدت تناسقا في تحرير البروجستيرون بشكل فعال وكما اسلف في الزجاج مغيرا المخاط المهبلي. واكثر من ذلك ابدى انتظام في تحرير وتاثير عالي تحرير الهومون ويعود ذلك الى نشوء مستودع في الغشاء المخاطي المهبلي ادى الى تقليل الجرعه واحداث تأثير مضاف. ومهما يكن استعمال الشكل النانوي عزز ايجابا فكرة بروتوكول سريري جديد لادارة ومساعدة في احداث الشبق ومزامنته وافراط الاباضه

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