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ESTROUS CYCLE SYNCHRONIZATION AND SUPEROVULATION EFFECT OF L-ARGININE CEFATOXIME PESSARY (ALCECO®) IN EWES (*PART II*)

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SUMMARY
This study compares superovulation efficiency and estrous cycle synchronization through treatment by L-arginine-Cefatoxime (AlCeco®) 200mg L-arginine per pessary and 500 mg Cefatoxime the estrus cycle-ovulation in ewewas investigating the induced cyclicity of ewe in same time and out of breading season, Estrous cycle synchronization, Superovulation performance, Combined with antibacterial to prevent complication of physiological changes by the following parameters Vaginal smear cytology and type of cells, Total estrous cycle length, Periods of estrous cycle phases, body weight, ovarian weight and ovarian to body weight ratio, Superovulation incidence, total number of follicles and number of sized follicles, total number of atretic follicles with their size, and number of corpora leutea. The results showed obvious estrous cycle synchronization prior superovulation indices as well as efficient at out of breading season. With increase of estrous windows facilitated chance of fertilization as well as hold a protectant effect by reducing bacterial counts. AlCeco®, a one of strategies was counted to synchronize and superovulate in ewe. Adjustments of ovarian functions appearance, as well as the doses and timing of their administration, were made to the protocols based on the ovulation response of the ewe.

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KEYWORDS: Ewe, ALCECO, Pessary, L-arginine, Bacteria, Synchronization, Ovary, follicle, Cefatoxime, antibacterial.

INTRODUCTION

Several attempting were performed to program animal fertility and upgrading accomplished economically. That done by ability to control animal's date of birth is usually of importance in animal breading of productive animal or animal husbandry. Accordingly, substantial research and development efforts have been directed at controlling the ewe reproductive cycle in order to produce lambs with actual birth dates.[1]

Ewes experience estrous cycle about 17-21 days in length, with some seasonal variation and except for a period of about 6 months in two season of activity. During the latter period, the ewe typically experiences an anestrous period. This period commonly ranges in cold hemisphere form about December, January and February and hotter hemisphere from about June, July and August.[2]

In the breading season the remarkable of correlation between prolonged days light periods and increase ewe ovulation rates led to the management of ewe breeding by "extending the photoperiod". Thus ovulation has been induced during pharmacologically maneuvers during anestrous or artificial induction. Thereby, extend the perceived cycling period overall year.[3]

In recent years, a number of studies determined the efficiency of multiple protocols and methods in the superovulatory protocols of ewes.[4,5] However, common notions reveled to the administration of steroidal hormones programs was performed without awareness to their short comes and uncontrolled control of estrous cycle stages as well as residual side effects, according to this fact it remains necessary to found alternative agents non-hormonal drug for created programed utero-ovarian cycle, have superovulation and estrous cycle synchronization with effectiveness of this treatment is equivalent at all stages of the estrous cycle.[6]

Aim of study, the present study relates to estrous cycle synchronization, ovulation control and superovulation by L-arginine and their pharmaceutical (AlCeco®) product to re-promotion fertility in out of breading season and

comparison and coincided with classical hormonal program on same effort in on ovarian function. L-arginine as nitric oxide donor complained Cefatoxime acts as prophylaxis antibacterial during L-arginine functional turnover effort on utero-ovarian changes.
In order to achieve set targets simultaneously or set at the time of the singular and those we:

1. Induced cyclicity of ewe in same time and out of breading season.
2. Estrous cycle synchronization.
3. Superovulation performance.
4. Combined with antibacterial to prevent complication of physiological changes.

MATERIALS AND METHODS

Management of Animals

The experiment was conducted out of breading season (December-January) in College of veterinary medicine, Thirty adult Iraqi local bread of ewe, between 1.5-2 year of age, body weight range 28-35 Kg were housed in circumstance of ordinary circadian rhythmic day light and temperature, Clinical examination for health fitness. The ewes were provided with water *ad libitum* and tested ewes were nourished a live weight maintenance ratio during breading period.

Estrous synchronization and superovulatory protocol

Estrous cycles of the animals were synchronization and superovulation by L-arginine-Cefatoxime (AlCeco®) 200mg L-arginine per pessary and 500 mg Cefatoxime. Three Pessaries, twice weekly for two weeks, intrauterine dosage form for group I; group II given on day one PMSG and followed by LH after two days of treatment[5], whereas, group III was served as control.

Parameters of study

The evaluation of estrous cycle synchronization and superovulation the following parameters were done:

1. Vaginal smear cytology and type of cells (%)
2. Total estrous cycle length (day)
3. Periods of estrous cycle phases (%)
4. Body weight, ovarian weight and ovarian to body weight ratio (%)
5. Superovulation incidence (%)
6. Total number of follicles and number of sized follicles (µm).
7. Total number of atretic follicles and number sized atretic follicles.
8. Number of corpora leutea.

Vaginal smear cytology and type of cells (%)

Time of estrous cycle phases detection were recorded, vaginal smears were taken every day to determine the changes in percent of different cells by stained 1% methylene blue. Cells were classified according to AI-Wahab and Khudyer.[6]

Total estrous cycle length (day) and periods of estrous cycle phases (%)

The exploration and scrutinize of influence of treatment on the estrous cycle period length, vaginal smears were taken from each ewes groups that examined daily each 6hrs, the cotton swab prepared from was inserted into the vagina and rotated then it was rolled on microscope slide, The vaginal smears were categorized into one of quadrate phases of estrous according to the cell types and shape.[7]

1. A number of leukocytes as well as "elongated nucleated epithelium" indicated proestrous.
2. Large cornified epithelial cells were exclusively found in estrous.
3. Metestrous was marked by a thick smear composed of equal numbers of nucleated epithelial cells and leukocytes
4. Smear consisting almost exclusively of leukocytes depicted diestrous.

The length of the estrous cycle and number of days spent at stage of the cycle evaluated for two consecutive estrous cycles.

Estrous cycle Length %

The phases of estrous cycle were determined by observing the vaginal smear in the morning at (10 to 11 hour) according to by Cooper *et al*.[8] The length of the estrous cycle and the number of days spent at each stage of the cycle evaluated as follows:

$$\text{The phase estrous cycle length (\%)} = \frac{\text{the estrous cycle phase period}}{\text{Total estrous cycle length}} \times 100$$

Body and ovarian weight and ovarian to body weight ratio (%)

The body weight ewes were check after two consecutive estrous cycles. The ewes were slaughtered and the ovaries excised, cleaned and weighted by sensitive balance.[9]

The ovarian to body weight ratio was extracted as follows:

$$\text{ratio of ovary to Body weight} = \frac{\text{ovarian weight}}{\text{Body weight}}$$

Superovulation incidence (%)

The number of superovulation ewes were counted and extracted as follows.[10]

$$\text{superovulaton\%} = \frac{\text{superovulated ewes numbers}}{\text{Total ewes numbers}} \times 100$$

Total number of healthy and atretic follicles and number of sized follicles (µm)

Ovaries of five ewes in each group were taken for follicular growing studies. The phases of estrous cycles of the five examined ovaries exhibited were in diestrus phase. The ovarian weight of the mice nearest to the

mean weight of the ovaries of respective group was selected. The ovaries were put in Bouin's fixative fluid[11], sectioned at 5-µm thickness, and stained with hematoxylin and eosin. Every one of successive sections of the ovary was reckoned for differents stages of development of follicles as describe by Moawad, *et al*.[12] and Bolon, *et al*.[13]

Follicles were classified according to Chen, *et al*.[14,15] into small, medium and large follicles. Various health sized follicles and atretic follicles were classified as described by Swartz and Mall.[16]

In this study, three classes of ovarian follicles were categorized using the comparative cross sectional diameter of the follicle as measured through the outside circumstances margins of the granulosa cell layers. These quantitative criteria represent a substantial generalization of an elaborated grading unit system recorded by Pedersen and Peters[17], with eight stages and several sub-stages to differentiate between primordial oocytes (Type 1) to antral follicle (Type 8) in mice.

1. Small follicles-(Pedersen and Peters Types 1-3b) involved of an extracted and isolated oocyte or an oocyte surrounded by a partial or unbroken layer of granulosa cells.
2. Medium (growing) follicles (Pedersen and Peters Types 4 - 5b) have an oocyte surrounded by multilayered, with granulosa cells as solid mantle of.
3. Large (antral) follicles - (Pedersen and Peters Types 6 - 8) were characterized by central oocyte and "fluid filled space bordered by number of granulosa cells".

By using these criteria, mean diameters of follicles have been measured at small follicles < 20 µm, medium follicles 20-70 µm and large follicles > 70 µm follicles in mice. Follicles displayed the nucleus of the oocyte were measured by using a calibrated ocular micrometer to avoid repeated counting. "The large diameter and diameter at the right angle to it were used to find a mean diameter for each follicle". A follicle was to be atresia or to regressing phase when two or more pyknotic granulose cells would be found in a single section or whether the oocyte showed signs of degeneration, such as fragmentation, absent of nuclear membrane, or reduce or thinning of cumulus oophorus as proposed by Osman.[18]

Number of corpora leutea

The structure follicles and corpus luteum histology section 5µm was calculated according to volume and morphology[19] this was determined by via ocular micrometer and counted certain structure component number of corpus and extracted according to formula.[20]

Number component = number of corpus luteum or follicles ÷ area

Bacterial count

Samples for microbiological culture were collected collectively from the vagina-cervix and uterus at all stage of the reproductive cycle. The swabs were collected pre and post treatment. Sterile plastic swabs were used for sampling. The outer surface of vagina was disinfected using alcohol 70% before sampling. After sampling, each swab was put into a tube containing 3 mL sterile 0.85% NaCl solution and shaking in order to release and liberate entire collected swabs material to the aqueous phase. These materials in tubes were centrifuged 3000 g for 10 min.

Residues were used as inoculate. Residue was inoculated onto 5% blood agar and MacConkey (Oxoid) agar Petri dishes were incubated in aerobic conditions for 24-48 h at 37C°. After incubation period, a colony was cultured and identified by biochemical tests, results were interpreted in accordance with the recording of Barrow and Feltham (1995) and Poveda (1998). We determined bacterial groups on the basis of a summary of the morphology of the bacterium, discrimination by use of Gram staining, dependence on aerobic conditions, morphology of the bacterial colony, chemical characteristics of the bacterial colony (production of hydrogen sulfide, metabolism of simple sugars) and spore formation.[38,39] The number of colony-forming units (CFU) per vagina was determined from the highest dilution at which colonies could be accurately counted. The number of bacteria was expressed as log10 counts of CFU per vagina. The total number of bacteria was calculated by totaling the counts of each bacterial group.

Statistics

Statistical evaluation between the control and treated data were subjected to analysis of variance (ANOVA). The F test was used for the analysis of follicular counts. A probability of <0.05 was assumed to denote a significant difference.[21]

RESULTS

The cellular changes during the estrus cycle after AlCeco® (L-agrinine - Cefatoxime) group rounded cell percent was higher ($p<0.05$) during proestrus and lower during estrus and metoestrus in comparison to control group. White blood cell percent was significantly ($p<0.05$) raised during estrous and metoestrus in AlCeco® groups as compared with control group. The hormonal set program treated group showed superior values than control but lower than AlCeco® treated group (table 1).

The estrous cycle day was non-significant $P>0.05$ unchanged in total length whereas, percentage of estrus phase period complained significant $P<0.05$ increase in AlCeco® treated group as compared with both control and Hormonal program treated group associated with significant $P<0.05$ reduce of percentages periods of other phases in AlCeco® treated group as compared with other; Table 2.

Table (1): Vaginal smear cytology during, percent of round squamous and white blood cells of estrous cycle phases epithelium in control L-arginine-Cefatoxime (AlCeco®)[1] and hormonal program[2] treated groups.

Types of cells in vaginal smear %				
		Round	**Squamous**	**White blood cells**
Protestors	Control	71.37 ± 0.95a	19.31 ± 0.32a	9.32 ± 0.40a
	AlCeco®	76.55 ± 2.33b	13.48 ± 1.50b	9.97 ± 0.85b
	Hormonal program	75.40 ± 3.50b	17.33 ± 0.82ab	7.27 ± 1.49c
Estrous	Control	16.61 ± 0.95a	75.38 ± 6.15a	8.01 ± 0.25a
	AlCeco®	6.94 ± 0.47b	78.12 ± 3.94b	14.94 ± 0.36b
	Hormonal program	9.14 ± 0.80c	77.94 ± 9.99ab	12.92 ±0.45b
Metestrous	Control	15.72 ± 1.09	75.65 ± 4.38	8.64 ± 1.04b
	AlCeco®	9.05 ± 78.94	78.94 ± 1.62	20.01 ± 0.50b
	Hormonal program	8.31 ± 1.12	76.83 ± 3.78	14.88 ± 0.61a
Diestrous	Control	80.36 ± 2.30a	10.80 ±0.49a	8.84 ± 0.32a
	AlCeco®	76.24 ± 1.98b	14.09 ± 0.96b	9.67 ± 0.77a
	Hormonal program	78.98 ± 0.88b	15.15 ± 0.58b	5.87 ± 0.46b

[1] (AlCeco®) L-arginine 200 mg Cefatoxime 500 mg per pessary.
[2]program of Hormone Values presented as mean ±SE error; Letters: (P < 0.05) vs. differences between treatment group and control group. N = 10 ewe.

Table 2: Total estrous cycle length and period of estrous cycle phases% of vaginal smear detection of estrous cycle phases epithelium in control L-arginine-Cefatoxime (AlCeco®)[1] and hormonal program[2] treated groups.

Groups	**Total estrous cycle length days**	**Period of estrous cycle phases%**			
		Proestrus	**Estrus**	**Metestrus**	**Diestrus**
Control	19.39±2.80 a	15.36±3.07 a	18.55±1.72 a	22.14±2.60 a	43.93±2.13 a
AlCeco®	19.82±2.90 a	9.69±1.23 b	69.87±7.01 b	10.93±0.69 a	9.51±0.12 b
Hormonal program	20.06±1.03 a	19.99±0.61 b	20.01±3.34 b	21.36±1.04 b	38.64±0.53 b

[1](AlCeco®) L-arginine 200 mg Cefatoxime 500 mg per pessary.
[2] Hormonal program Values presented as mean ±SE; Letters: (P < 0.05) vs. differences between treatment group and control group. N = 10 ewe.

Table 3 displayed significant P<0.05 increase in body weight, ovarian weight and ovarian to body weight percentage in AlCeco® treated group as compared with both control and hormonal program treated groups.

The incidence of superovulation revealed to noticeable increased in AlCeco® treated group as compared with hormonal program group significantly P<0.05, where, no changes occur in control group; Table 4.

Table 5 showed significant P<0.05 increase in total number of ovarian follicles and all classes of follicular size in both AlCeco® and hormonal program treated groups as compared with control group. Whereas, significant P<0.05 drop in total atretic ovarian follicles number and numeral percentage of medium and large follicles in both AlCeco® and hormonal program treated groups as compared with control group Table 6. Finally the corpus leutum percentage in table 7 was significant P<0.05 superior value in both AlCeco® treatment and hormonal program groups comparable with control value of control group.

The total number of bacteria was influenced by the estrous cycle. The total number of bacteria during estrus was significantly p<0.05 higher than that during other stages of the estrous cycle in all ewes groups, whereas the AlCeco® treated group upset values of bacterial numbers as compared with control and maximal in hormonal program treated group of ewes.

Table 3 body weight and ovarian weigh to body weight ratio in control, L-arginine-Cefatoxime (AlCeco®)[1] and hormonal program[2] treated groups.

Groups	Body weight kg	Ovarian weight g	Ovarian weight to body weight ratio %
Control	35.62 ± 1.82 a	37.21 ± 0.79 a	0.104 ± 0.019 a
AlCeco®	40.15 ± 2.33 b	79.38 ± 0.40 b	0.191 ± 0.017 b
Hormonal program	40.27 ± 1.03 b	80.05 ± 2.94 b	0.198 ± 0.028 b

[1](AlCeco®) L-arginine 200 mg Cefatoxime 500 mg per pessary.
[2]Hormonal program Values presented as mean ± SE; Letters: (P < 0.05) vs. differences between treatment group and control group. N = 10 ewe.

Table 4: superovulation incidence in control, L-arginine-Cefatoxime (AlCeco®)1 and hormonal program2 treated groups.

	Control	AlCeco®	Super ovulation program
Superovulation incidence %	0.0 ± 0.0 a	95.48 ± 3.41 b	86.37 ± 2.21 c

[1](AlCeco®) L-arginine 200 mg Cefatoxime 500 mg per pessary.
[2]Hormonal program Values presented as mean ± SE; Letters: (P < 0.05) vs. differences between treatment group and control group. N = 10 ewe.

Table 5: Total number and follicular size in control, L-arginine-Cefatoxime (AlCeco®)[1] and hormonal program[2] treated groups.

Groups	Number of follicles according to size classification (diameter)			Total number of follicles
	Small < 20 µm	Medium 20-70 µm	Large > 70 µm	
Control	26.5 ± 3.85 a	4.39 ± 0.85a	0.89 ± 0.02 a	31.67 ± 0.21a
AlCeco®	30.62 ± 0.55 b	11.92 ± 1.78 b	9.85 ± 0.16 b	50.39 ± 0.21b
Superovulation program	32.74 ± 1.03b	11.20 ± 0.11b	9.00 ± 1.02b	52.94 ± 1.69b

[1](AlCeco®) L-arginine 200 mg Cefatoxime 500 mg per pessary.
[2]Hormonal program Values presented as mean ± SE; Letters: (P < 0.05) vs. differences between treatment group and control group. N = 10 ewe.

Table 6: Ovarian atretic follicles number in control, L-arginine-Cefatoxime (AlCeco®)[1] and hormonal program[2] treated groups.

Groups	percentage of atretic follicles according to size classification (diameter)		Total number of atretic follicles
	Medium 20 - 70 µm	Large > 70 µm	
Control	58.4 ± 2.61 a	94.72 ± 3.28 a	3.01 ±1.12 a
AlCeco®	21.28 ± 1.99 b	60.75 ± 6.45 b	0.15 ± 1.31 b
Superovulation program	19.51 ± 1.03 b	60.25 ± 2.94 b	0.18 ± 0.01 b

[1](AlCeco®) L-arginine 200 mg Cefatoxime 500 mg per pessary.
[2]Hormonal program Values presented as mean ±SE; Letters: (P < 0.05) vs. differences between treatment group and control group. N = 10 ewe.

Table 7: number of corpus leuteum in control, L-arginine-Cefatoxime (AlCeco®)[1] and hormonal program[2] treated groups.

	Control group	AlCeco®	Super ovulation program
Corpora leutea number	2.1 ± 0.26 a	17.17 ± 0.26 b	16.99 ± 1.30 b

[1](AlCeco®) L-arginine 200 mg Cefuroxime 500 mg per pessary.
[2]Hormonal program
N = 10 ewe
Values presented as mean ± SE
Letters: (P < 0.05) vs. differences between treatment group and control group.

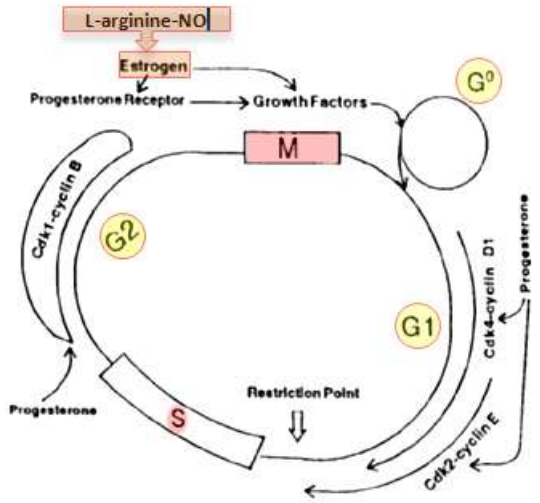

Figure (1): Proposed model for progesterone-dependent endometrial cell proliferation cycle viewing potential control points for cell cycle progression. Cells are driven into S and M phases by the formation of cyclin–cdk complexes. Progesterone may motivate the synthesis of cyclins in G1 and G2 phases of the cell cycle. Activation of the complex requires kinase binding to cyclin, phosphorylation, and dephosphorylation. Upregulation setpoint of L-arginine-nitric oxide induced estrogen may be needed for the synthesis of growth factors and progesterone receptor.

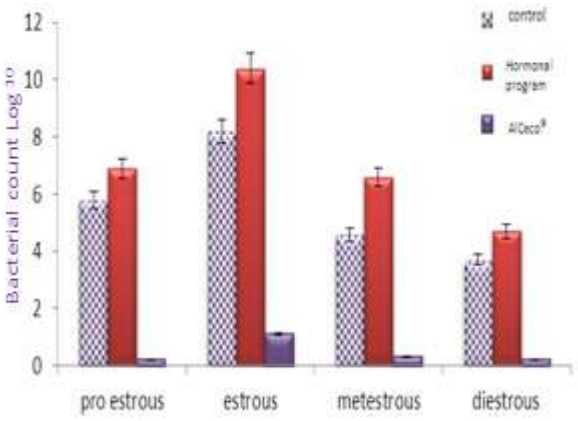

Figure 2: Relation between vaginal bacteria and estrous cycle in control, L-arginine-Cefatoxime (AlCeco®)[1] L-arginine 200 mg Cefatoxime 500 mg per pessary and hormonal program treated groups, the total number of bacteria was determined during each stage of the estrous cycle proestrus, estrus, metestrus, and diestrus. Mean values are expressed on a log^{10} scale.

Values presented as mean ± SE; Letters: ($P < 0.05$) vs. differences between treatment group and control group. N = 10 ewe.

DISCUSSION

Recent studies had shown that nitric oxide donor treatment modulation of the intrauterine environment and endocrine status, maternal utero-ovarian cycling and rhythms are major factors affecting the performance, ovulation and estrous cycle regulation.[22] Among several drugs and hormonal maneuvers were done to control estrous and superovulation without awareness to their adverse effects and fluctuation performance and some failure to eliminate restrictions of "seasonal photoreceptive breeders" and to remove the confounding variability of cyclical changes in ovarian steroids, amino acids had newly received ample attention, because they are the building blocks of proteins and regulators of key metabolic pathways.[4,23] Accordingly, ovarian and uterine tissue contain large amounts of free amino acids specially L-arginine, suggesting their role in derived Nitric oxide.[24,24] In addition, uterine fluid are rich in the arginine and their end or precursor product of amino acids, comprising arginine, citrulline, ornithine and glutamine, during estrus phase.[25 25–27]

The cyclical uterine parameters were examined in this study and their correlations to intrauterine L-arginine, in conjunction through significant changes among each estrous cycle stages (table 1). L-arginine derived nitric oxide treated group showed superior hyperplasia in squamous cells of endometrium than control group significantly and produced non statistically than hormonal program in estrous phase may be due to direct changes correlate with the natural levels of circulating hormones. Determining the stage of the ewe estrous cycle by vaginal smear analysis requires interpretation of cell types present in the smear. To minimize intra-stage variations, we collected uterine tissue that represented the earliest transition from one stage to the next. As a complement to vaginal cytology, we also measured serum Estrogen (E2) and Progesterone (P4) levels for every ewe, which permitted testing of correlations irrespective of estrous stage.[29]

The L-arginine-nitric oxide had provocation effect to estrogen synthesis and estrogenic factors modulators as well as estrogen like factor (,31 30واسراء وفريال شيماء), creating upstairs levels of estrogen and E_2 receptor upregulation.[32] Estrogen has a major concern regarding the effectiveness of the estrogen-dependent proliferation of endometrium, which required essential a minority amount of estradiol is prerequisite to initiate endometrium stromal cell proliferation; Savouret *et al*.[33] confirmed there had no direct effect of estrogen on cell cycle progression in uterine endometrium cells has been established. Therefore, our explanation of the existing evidence is that estrogen functions in two important aspects of stromal cell proliferation. First, L-arginine-nitric oxide stimulated estrogen system may be necessary in intact animals to motivate and stimulate the creation of progesterone receptor rather than to direct cell cycle progression (figure 1). "Consistent with this interpretation, estrogen induces transcription of the

progesterone receptor via an intragenic response element.[34],,

Kraus and Katzenellenbogen[34] revealed to that estrogen is required for progesterone processes sequel upshot in uterine tissue due to the increase progesterone receptor content, that proliferating uterine stromal cells motivate both the A and B subtype of the progesterone receptor.[35]

Moreover L-arginine-Nitric oxide had positive modification of increase progesterone receptors in uterine tissue and the result in table ( ) serum hormonal levels showed significant $P<0.05$ slightly increase progesterone as compared to control group that give correlated interpretation to hypothesized mechanism of estrogen-progesterone promotion of cell cycle phases, that made dominancy of squamous cells in estrous phase. Alternatively, progesterone receptor synthesis may be the outcome of a direct link between the estrogen receptor to an insulin-dependent mechanism[36], Furthermore, Nitric oxide provoked insulin synthesis[37] that triggered and maintenance of estrogen receptor through increased receptor life spam associated with upregulation.[36]

The second important function of estrogen that may be induced by L-arginine-Nitric oxide was presumably to stimulate the production of uterine growth factors system. That proliferating cells express a single form of fibroblast growth factor receptor-1 and progesterone inhibited growth factor and upset cell proliferation, uterine growth factors levels increase in response to estrogen[37] that speculation presumably demonstrated the reduction in proliferative processes of uterine tissue in luteal phase in AlCeco® treated group and hormonal group as compared to control group.

Table 2 displayed the elongation of estrus phase period at the expense of the other in the duration of estrous cycle phases periods in AlCeco® that give an impression to large window was open several time of fertilization attempting. AlCeco® containing L-arginine obviously modulated hormonal level Rhythms might be attributed to first: direct alteration of ovarian tissues recreated steroidal hormone and/or second indirect modification and modulation of hypothalamic-pituitary-ovarian axis.

First, insertion of intrauterine L-arginine pessary was accelerating function of reaction mediated NOS properties and promotes K12 forward reaction yield of nitric oxide (Mitchell *et al*., 2002). These evidences reveled to the L-arginine derived nitric oxide AlCeco® dominance increased estradiol secretion by hyperplasia and hypertrophy of ovarian granulosa cells sent decision extend estrus phase windows period (Voorhis, *et al*., 1994) and this had normal rheostat like system proved activity under usual dominance positive and upturn threshold of negative feedback hormonal regulatory machinery hypothalamic main switch guide of ovarian rhythmicity. This result was coincided with our finding result of hormonal level of estrogen (Table) revealed to more pronounce increase of their values in estrous of AlCeco® treated group.

Secondly the changes of endometrium dramatic turnovers presumably indirect due to extra-follicular regulation over modulation or modification of hypothalamic-pituitary-ovarian axis of L-arginine, Moretto, *et al*. (1993) conveyed the GnRH neurons at median eminence are under the active regulatory effect of L-arginine-NO, at the matching time increase the release of the hypothalamic hormone and during the ovogenesis (Knauf *et al*., 2001). This fiction provide clear evidence anion NO potently arouses GnRH by triggering a heme containing enzyme, guanylate cyclase, which in turn leads to increased production of c.GMP forward GnRH release (Ojeda and Urbanski, 1994 and Dhandapani and Brann 2000).

This episode of GnRH stimulatory effect of NO for GnRH had associated with sensitivity diminution of GnRH-gonadotrophin axis to the inhibitory feedback effect of estrogen (Reynoso *et al*. 2002).

Furthermore, L-arginine derived NO provoked follicle growth via a direct mechanism even in the absence of systemic influences, upshot upsurge secretory functional cells groups in Graffian follicles yielded estrogen sophisticated levels and overstated the estrous phase Mitchell, *et al*. (2002).

The results in table 3 indicated to amplification in ovarian weight and ovarian to body weight ratio that may be to several conventional systemic and locally turnovers under AlCeco® intrauterine pessary primary presumably attributed to: 1. the direct positive relationship between NO concentrations in follicular growth and negatively with “programmed follicular cell death (apoptosis). Folliculogenesis includes the contribution of both growth at the follicle and apoptosis. Nitric oxide regulates both of these processes Sugino *et al*. (1993)”. 2. Increase blood volume by direct achieved by NO-c.GMP vasodilatation of veno-arterial blood vessels. 3. Extravasation of blood components into the pericapillary space (Brännström *et al*., 1993) and formation of edema like fluid in the ovarian stromal tissue (Bjersing and Cajander, 1974). 4. Escalation of follicular size occurs due to upturn and upwelling estrogen concentrations, and ovarian artery and intra-ovarian blood flow (Anteby, *et al*. 1996) further more L-arginine derived nitric oxide had angiogenic promoting activity by vasodilator pressure activity and activated angiogenic growth factors to perfused angiogenesis, this increase blood volume and endpoint increase ovarian weight.

The folliculogenesis parameters in ovarian loaded by AlCeco® dosed intrauterine L-arginine complain loftier value than control group (table 4, 5 and 6) that attributed to facts; the ovary is a highly vascularized organ (Abisogun *et al*., 1988). L-arginine enhance and demined the growth of the ovarian follicles through; “encouraged

the capillaries in the theca interna, which bordering the vascular antrum/granulosa cells compartment proliferate and transition with anastomose to procedure a basket-like structure (Murakami, *et al*., 1988)". Shortly after the preovulatory surge of LH, upstairs ovarian blood flow intensifications (Janson, 1975 and Brännström, *et al*., 1998) and there is a marked dilatation of the vessels bordering and invasive the ovulating follicles (Kranzfelder *et al*., 1992). These changes in the ovarian blood flow are accompanied by signs of amplified permeability in the follicular microvasculature (Okuda *et al*., 1983), extravasation of blood cells and constituents into the pericapillary compartmental space (Brännström *et al*., 1993) and formation of edema like fluid in the ovarian stromal tissue (Bjersing and Cajander, 1974) This finding is coincided with grossly appearance (figure 1) and histological architectures of ovary in AlCeco® treated group.

Whereas, the atretic follicles were upset calculated values in AlCeco® treated intrauterine might be due to several lines of evidence support the role of L.arginine nitric oxide pathway in the follicular development and ovulatory process. In the present suggested that L-arginine releasing NO may function as a vasodilator promoting the increased ovarian blood flow that is important for ovulation under follicular pressure theory (Andronowska, *et al*., 1999) which was attributed to suggested reduction of follicular atresia results, that means the follicles shift to right as an ovulatory process more than atretic changes. Numerous factors promotes activity processes outcome of L-arginine, may be occurs due to; ovarian NO is important for optimum prostaglandin synthesis during the periovulatory period (Jana, *et al*., 2000). It is also likely that oocyte NO may triggering and acts as a signal for somatic cells to properly functional modify the theca and tunica albuginea of the follicle wall necessary for the oocyte to exit the ovary at ovulation. Furthermore, (Juengel, *et al*., 1993) presumably attributed the increase number and size of follicles to L-arginine derived releasing NO may affect oocyte meiotic maturation by binds to and activates soluble guanylyl cyclase, derived c.GMP levels in target cells. c.GMP has been localized in the ovarian granulosa cells (Brunswig, *et al*., 1997). In other hand suggested that c.GMP lowers the c.AMP level by triggering oocyte c.AMP-phosphodiesterase (c.AMP-PDE) and thus permitting oocyte maturation to continue (Brunswig, *et al*., 1997). The evidence implicating c.AMP, c.GMP and c.AMP-PDE in meiotic maturation combined with the effect of NO on oocyte maturation raise the possibility that NO regulates oocyte cAMP level either by increasing c.GMP synthesis and/or by stimulating c.AMP-PDE activity during the transition from metaphase I to metaphase II of meiosis. Extra causes may be the nitric oxide play a role in follicular atresia and apoptosis, which found that trigger amount of NO may prevent apoptosis and degenerative change known as atresia (both pro and anti-apoptotic properties) at varying stages of follicular development, and increase crowded large follicles and secreatory cells and their count (table) (Chen, *et al*., 2005).

Besides, specific relationship may explain the indirect effect of L-arginine-nitric oxide on follicular development through augmenting the patterns on follicular units of follicle and increase undergo magnification, FSH hormone induced superovulation and reduced atresia follicles (Hattori *et al*., 2001). That encouraged idea impression increase FSH surge in intensifies level multiple incidence of follicular growth and maturity and rise superovulation incidence during intrauterine L-arginine loading dose as compared with control group and hormonal program.

Inspection of anatomical structure of utero-ovarian crossly showed enlargement of ovarian size and flocculated in a lot of number and there are evidence of superovulation showed in a multiple ovulated oocyte in atretic that indictable to maintain upper function and propulsive of ovary with consequence of their physiological cycling Figure (1).

Table (7) displayed the corpus leuteum percentage was higher value in both AlCeco® treatment comparable with hormonal program groups and control group that may be attributed to involvement NO in the regulation of corpus luteum (CL) function and determinate of lifespan (Motta, *et al*., 2001), Furthemor, Hurwitz, *et al*. (1997) suggested that functional L.arginine-nitric oxide reaction unit stimulates both glutathione, a major antioxidant and progesterone production, thus favoring the adjusted and regulate of CL; NO, together with PGE2, seems to act through its effects on vasculature and proteolytic processes.

So Dong, *et al*. (1997 and 1999) they speculated that NO could initiation of ovulation and CL regulation by prostaglandins, thus maintaining adequate levels of Leutolytic factors and possibly involved in the control of luteal vascularization, but the precise mechanisms by which it exerts its effects remain to be elucidated. That achieve endpoint reduction of diestrous length and progesterone levels associated with reduction of absolute volume density of corpus luteum (tables 2). L-arginine as NO donor was, for the most part, luteolytic, in order to the NO donor caused immediate large increases of Leukotrine (LTC4) and PGF2α as leuteolytic effects (Lei *et al*., 1991).

An examination assumed to identify and recognize the vaginal flora of cycling ewes suffering from estrous cycle change during hormonal maneuvers and L-arginine pharmaceutics pessary act as predisposing factor for risk facilitate bacterial invasion, revealed that intrauterine AlCeco® pessary reduced the vaginal bacterial counts, Perusal of the antibiogram pattern revealed that ~90% of the bacterial isolates from vaginal swap of AlCeco® treated ewe were sensitive to Cefatoxime containing pessary with L-arginine that is active against both Gram-

positive and Gram-negative bacteria (Loebstein *et al*., 1998). In nutshell it may be inferred that when antibiotics are to be used to prevent or prophylaxis genital tract infections during immune turnover accompanying with hormonal fluctuations at estrous cycle Shifts, extreme care used the judicious this antibiotic can cause serious detrimental part to achieves safeness immune suppression during steroidal hormone dominant with awareness combined with vaginitis or endometritis in time of breeding like seen in hormonal program.

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