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EFFECT OF SOME METABOLIC MEDIATORS TREATMENT DURING POSTPARTUM ON MILK PRODUCTION AND REPRODUCTIVE PERFORMANCE OF PRIMI- AND MULTI-PAROUS FRIESIAN COWS.

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ABSTRACT

To evaluate the effect of some metabolic mediators such as insulin or/and L-carnitine on milk yield, reproductive performance of premi- (P1) and multi-parous (P2) cows during postpartum (PP), 40 Friesian cows (20 P1 and 20 P2) were divided into 4 similar groups (10 animals in each, 5 P1 and 5 P2). Animals in the 1st group (T1) were considered as a control, while those in the 2nd group (T2) were weekly i.m. injected with 40 IU insulin/100 kg LBW. Animals in the 3rd group (T3) were weekly administrated with oral dose of 1 g L-carnitine (LC)/100 kg LBW, while those in the 4th group (T4) were administrated with an i.m injection of 40 IU insulin plus oral dose of 1 g LC/100 kg LBW. All treatments were within 7-10 days after calving up to first service. All animals were kept under the same management and feeding system. All cows were milked twice daily by milking machine. Milk yield was weekly recorded and milk composition was monthly determined for 4 month-lactation. Animals in heat 40-45 days postpartum were artificially inseminated and pregnancy diagnosis was carried out 45 d post-service. Blood samples were collected at 0, 1, 2, 3 wk of treatment, estrus and pregnancy for progesterone (P_4) assay in blood serum. Different reproductive measurements were recorded for 4 month-PP. The obtained data showed that average weekly milk yield (AWMY) was higher (P<0.05) in T4 than in T1, but did not differ from that in T2 and T3 only during the 2nd month of lactation. T2 and T3 insignificantly increased AWMY as compared to T1. Average daily milk yield (ADMY) or total milk yield (TMY) increased (P<0.05) in T2, T3 and T4 by about 13, 14 and 15% as compared toT1, respectively. AWMY, ADMY and TMY were not affected by parity. Milk composition was not affected by treatment at different lactation months. Percentages of protein, solids not fat and total solids in milk only during the 2nd month of lactation were higher (P<0.05) in P2 than in P1. Average interval from treatment to estrus (ITE) or to conception (ITC) and number of doses required for conception were the shortest inT3. T2 and T4 showed nearly similar values. T3 showed shorter (P<0.05) postpartum first estrus (PPFEI) and service (PPFSI) intervals than T1 (58.9 and 65.2 vs. 86.9 and 95.3 d, respectively). T3 showed high (P<0.05) conception rate (CR, 78%), least number of services per conception (NSC, 1.33), lowest days open (DO, 81.4 d) and the shortest calving interval (CT, 368 d), while T4 showed the highest (P<0.05) CR (90%). ITE, NTC, ITC, PPFEI, PPFSI, DO and CI were lower (P<0.05), while NSC and CR was greater (P<0.05) in P2 than in P1. Serum P4 concentration at 0, 1, 2 and 3 wk of treatment, 1st estrus, and estrus of conception was not affected by treatment. Only, serum P4 concentration at pregnancy was higher (P<0.05) in T4 than in T2 and T3, but did not differ from that in T1. Serum P4 concentration was higher (P<0.05) in P2 than in P1 only at estrus of conception and pregnancy.In conclusion, administration with an i.m injection of 40 IU insulin plus oral dose of 1 g LC/100 kg LBW during early postpartum period may consider successful treatment for improving milk yield and reproductive performance of lactating Friesian cows (primi- and multi-parous) under the experimental conditions.

Keywords: Cows, parity, insulin, L-carnitine, milk, reproduction, progesterone.

INTRODUCTION

Poor fertility in repeat breeder cows is a multi factorial problem. Life time milk production of cows depends upon re-occurring pregnancy because pregnancy initiates and renews the lactation cycle (Lucy, 2001). One of the goals of dairy management programs is to achieve short postpartum period and yielding one calve each year. Delaying the resumption of postpartum ovarian activity increases days open and postpartum period and decreases longevity of dairy cows.

Reduced diameter of the first dominant follicle, slower growth rate and decreased secretion of estradiol resulted in a delayed first ovulation postpartum (Sheldon *et al*., 2002). Negative energy balance (NEB) is the situation in which intake of feed energy is less than the output of energy from the body. This lack of energy can impair the development of the ovarian follicle and corpus luteum (CL) and result in less progesterone in serum (Spicer *et al.,* 1990; Villa-Godoy *et al*., 1988 and 1990).

Concentrations of IGF-I in the serum of cattle are decreased during restriction of dietary energy (Spicer *et al*., 1992) and during the NEB associated with early lactation (Sharma *et al*., 1994). Thus, IGF-I could serve as the mediator of the adverse effect of NEB on the CL. Spicer *et al*. (1990) observed a positive correlation between the concentrations of IGF-I and progesterone in the serum of lactating cows. However, the concentration of IGF-I in serum is only one measurement of a complex system (McGuire *et al.,* 1992).

Application of insulin to modulate reproduction is fairly recent concept (Suguna *et al*., 2009). Growth factors and metabolic hormones like somatotropins, insulin and IGF-1 had received attention in regulation of ovarian function (Simpson *et al*., 1994; Totey *et al*., 1996). Insulin also plays an important role in follicular growth through its direct stimulatory effects on granulosa cell estradiol production and indirect stimulatory effects via amplification of gonadotropin action (Gong *et al*., 1994). Moreover, insulin injection in cows increased the steroidogenic capacity, the diameter of the largest follicle (Simpson *et al*., 1994), and the ovulation rate in response to a superovulatory program (Harrison and Randel, 1986). Evidence also shows that, in buffalos suffering from summer acyclicity, insulin pretreatment can increase the diameter of the largest follicle and the estrous induction rate (Ramoun *et al*., 2007). Based on these information, it is possible that insulin injection in the moment of progesterone withdrawal in postpartum cows can improve the final follicular development, improving ovulation and, consequently, the pregnancy rate, administration insulin induced a positive effect on ovarian response in nulliparous goats (Peixoto Pinheiro *et al*., 2012), pretreatment with insulin for 3 days before GnRH injection increases the size of the largest follicle and the oestrous induction rate in buffaloes suffering from summer acyclicity (Ramoun *et al*., 2007),

L-carnitine, a vitamin-like quaternary ammonium compound is vitally important and endogenously synthesized from lysine and methionine in liver and kidneys (Rebouche and Engel, 1980). L-carnitine plays an important role

in the production of energy via mitochondrial β- oxidation in cells (Ohlenschlaeger and Berger, 1990). Carnitine is shown to have important functions in some metabolic processes such as oxidation of long-chain fatty acids (Oven *et al*., 1996), regulation of ketosis (Mc Garry *et al*., 1975), support of the immune system (Famularo and De Simone, 1995) and enhancement of the antioxidant system (Arockia Rani and Panneerselvam, 2001; Marasli *et al*., 2005) and improvement of reproduction. L-carnitine administrations increased glucose (the main source of energy) concentration in advanced pregnant Damascus goats with multiple pregnancies (Kacar *et al*., 2010).

Researchers reported that L-carnitine regulates metabolic processes in high yielding lactating cows and also ewes in an advanced stage of pregnancy. Recent studies indicate that while supplemental L-carnitine in the diet is not required, its use is recommended in domestic animals especially in cattle to increase performance and to support medical treatment (Citil *et al*., 2009). Supplemental carnitine in ruminant effected selected biochemical parameters such as triglycerides, cholesterol, urea, glucose, triiodothyronine (T3) and thyroxine (T4), which are indicators of energy metabolism (Citil *et al*., 2009). The effect of L-carnitine could be associated with stimulation of lipid metabolism through transfer of acyl groups across the mitochondrial membranes (Oven *et al*., 1996). A limited number of studies have dealt with effects of supplemental carnitine on metabolism and performance parameters in healthy ruminants (Chapa *et al*., 2001; Carlson *et al*., 2006; Pancarci *et al*., 2007).

Therefore, the aim of the present study was to evaluate the effect of some metabolic mediators such as insulin or/and L-carnitine on milk yield, reproductive performance of primi-parous and multi-parous Friesian cows during postpartum period.

MATERIALS AND METHODS

This study was carried out at Sakha Experimental Station, belonging to Animal Production Research Institute, Agricultural Research Center, Ministry of Agriculture, Egypt, in cooperation with Animal Production Department, Faculty of Agriculture, Mansoura University, during the period from September 2011 to February 2012.

Animals and management:

This study was conducted on 40 Friesian cows (20 primi- and 20 multi-parous cows) during postpartum period. Average Live body weight was 400±8.1kg for primi-parous cows and 552 kg for multi-parous cows (2-7 parities and 2.5-3.5 body condition score). All experimental animals had normal parturition with placental drop duration from 8 to 12 hours and did not develop any reproductive disorders after calving.

During the experimental period, animals were submitted to the ordinary system applied at the station, being indoors all over the year and were fed on diet that met both maintenance and production requirements. The type of offered feed differed according to the season, being green feeding system

including concentrate feed mixture (CFM) plus fresh Egyptian berseem (*Trifolium alixandrinum,* 2nd-4th cut) and rice straw (RS).

Experimental design and treatments:

The experimental animals (n=40) were divided into four similar groups, according to their LBW, parity and milk production, 10 animals in each (5 primi- and 5 multi-parous cows. All cows were fed the same diet. Animals in the 1st group (G1) were considered as a control group. Meanwhile, those in the 2nd group (G2) were weekly injected intramuscularly with 40 IU insulin/100 kg LBW. Animals in the 3rd group (G3) were weekly administrated with oral dose of 1 g L-carnitine (LC)/100 kg LBW, while those in the 4th group (G4) were administrated an i.m injection of 40 IU insulin/100 kg LBW plus oral dose of 1 g LC/100 kg weekly live body weight in oral dose for each cows weekly. All treatments were within 7-10 days after calving up to first service.

Milking and suckling system:

During the 1st week postpartum, calves in all groups were left with their dams for 3-4 days to receive the colostrums, and then they were artificially suckled until weaning. Thereafter, all cows were milked twice daily at 7:00 h and 17:00 h by milking machine and milk yield was weekly recorded. Also, milk composition was monthly determined using milko-scan.

Experimental procedures:

Resumption of ovarian and estrous activities:

Resumption of ovarian activity and cyclicity were assessed by examination of ovaries, rectally, ultrasonography and plasma progesterone (P4) profile.

Animals were individually observed twice daily (6.00 and 16.00 h) by herdsmen for estrous detection. All cows either those showed estrous signs or sustained anestrus were examined rectally twice weekly for detection of ovarian structures (follicles or CL). Also, transrectal examination by ultrasonography was conducted just after rectal palpation to record ovarian structures. Animal was considered to resume ovarian activity when blood serum P4 level exceeds 1 ng/ml.

Service and pregnancy diagnosis:

Artificial insemination was used as a method of breeding in the farm under the current study for all cows in heat 40-45 days postpartum. For each cow, the date of service was recorded and thereafter followed up for estrus return 21 days later. Non-return animals were ultrasonoghraphy examined 45 days after the first breeding for pregnancy diagnosis and in any doubtful case, the examination was repeated 2 weeks later to determine conception rate.

Blood samples:

Blood samples were obtained from the Jugular vein puncture from each animal at 0, 1, 2, 3 week, estrus and 120 days postpartum for progesterone (P_4) assay in blood serum. The collected blood samples were left in an inclined position for 3-4 h and then centrifuged at 3000 rpm for 10 minutes for serum separation, which was stored at -20 ^{o}C until analyses.

Reproductive data:

The effect of treatments in the present study on reproductive performance of cows was evaluated in the light of the following reproductive measurements:

- *Postpartum first estrus interval (PPFEI):* the interval from calving to first signs of estrous activity determined visual observation and confirmed by P4 serum concentrations.
- *Postpartum first service interval (PPFSI):* the interval from calving to first service of cows in heat.
- *Service period (SP):* the period from the first service to fertile service.
- Days open (DO): the interval from calving to fertile service.
- Number of services per conception (NSC): average number of services required for conception for each group within 60, 90, 120 day.

Treatment to first service interval (TSI): the interval from treatment first to first service of cows in heat.

Conception rate (CR): the total number of conceived cows/the number of served ones.

Calving interval (CI): The interval between the current calving and the following calving.

Number treatment to first service (NTS): average number of treatment required for service for each group.

Treatment to first conception interval (NTC): the interval from treatment to first conception.

Progesterone assay:

Direct radioimmunoassay technique (RIA) was performed for determination of serum P4 concentration using ready antibody coated tubes kit (Diagnosis Systems Laboratories Texas, USA) according to the procedure outlined by the manufacturer.

Statistical analysis:

The obtained data were statistically analyzed according to Snedecor and Cochran (1982) using computer programme of SAS (2004) to test the effect of treatment, parity and their interaction. The significant differences were carried out using Multiple Range Test of Duncan (1955). Conception rate was analyzed using Chi-square test.

RESULTS AND DISCUSION

Milk production:

Milk yield:

Data in Table (1) showed that average weekly milk yield (AWMY) of cows during 120 d of lactation period was affected significantly ($P<0.05$) by treatment only during the 2nd month of lactation, being higher in T4 than in T1 (control), but did not differ significantly from that in T2 and T3. Also, cows in T2 and T3 increased AWMY as compared to T1, but the differences were not significant. However, AWMY was not affected significantly by treatment during the 1st, 3rd and 4th month of lactation, although there was a tendency of higher AWMY in treatment groups than in the control one.

When milk yield was expressed as average daily milk yield (ADMY) or total milk yield (TMY) during the same lactation period, cows in all treatment groups showed significantly (P<0.05) higher ADMY and TMY than those in the control group by about 13, 14 and 15% for T2, T3 and T4, respectively. Such trend revealed that milk yield slightly increased with LC as compared to INS, but their combination was superior both treatment, indicating synergistic effect of both treatments on milk production than that of each treatment alone (Table 1).

Table (1): Effect of treatment on average weekly milk yield of cows at different lactation months.

Lactation month	Treatment (T)			
	T1(Control)	T2 (INS)	T3 (LC)	T4 (INS+LC)
Average weekly milk yield (kg):				
1 mo	89.2±5.65	102.7±10.40	102.2±4.69	98.4±3.69
2 mo	93.6±4.10^{b}	108.9±5.83^{ab}	106.0±4.70^{ab}	114.7±3.33^{a}
3 mo	100.4±7.06	106.2±8.09	108.0±3.89	111.8±6.80
4 mo	93.7±5.44	109.1±7.11	112.4±6.21	107.6±9.15
ADMY (kg)	13.46±0.67^{b}	15.25±0.76^{a}	15.31±0.59^{a}	15.45±0.63^{a}
TMY (kg)	1615.2±50.65^{b}	1829.6±60.34^{a}	1836.7±42.00^{a}	1853.6±45.82^{a}

a and b:Means denoted within the same row with different superscripts are significantly different at P<0.05). ADMY: Average daily milk yield. TMY: Total milk yield.

As affected by parity, AWMY, ADMY and TMY was not affected significantly by parity of cows, although they tended to be higher in multi- than in primi-parous cows during all lactation months. Also, the effect of interaction between parity and treatment was not significant in milk yield parameters, indicating the highest AWMY, ADMY and TMY of multi-parous cows treated with INS plus LC as compared to other treatment and control groups (Table 2).

The improvement in milk yield of Friesian cows (T3) with LC was previously recorded by Freemaut *et al.* (1993) on sows. Also, Noseir, *et al*. (2003) reported that milk yield (7% fat corrected milk) was significantly (P<0.01) higher during the first 120 days of lactation in buffalo cows receiving LC when compared with control group.

Table (2): Effect of parity on average weekly milk yield of cows at different lactation months.

Lactation month	Parity (P)		Interaction
	Primi-parous cows	Multi-parous cows	T x P
Average weekly milk yield (kg):			
1 mo	94.7±3.66	101.6±5.48	P< 0.057
2 mo	104.4±2.79	107.2±6.14	P< 0.146
3 mo	108.7±3.57	104.5±5.52	P< 0.577
4 mo	103.9±3.06	107.5±6.60	P< 0.736
ADMY	14.70±0.23	15.03±0.68	P< 0.168
TMY	1764.4±24.13	1803.3±51.39	P< 0.168

ADMY: Average daily milk yield. TMY: Total milk yield.

Freemaut *et al.* (1993) suggested that the positive effects of LC on milk production were probably resulted of better energy utilization. In this way, Harmeyer and Schlumbohm (1997) and Harmeyer (2001) reported that L-carnitine has a number of functions, such as transforming fatty acids into energy, preventing ketosis, carrying ATP from mitochondria to cytosole, increasing milk rate, supporting immune system. Moreover, Noseir *et al*. (2003) found that L-carnitine supplementation led to a significant increase in milk yield due to improvement in body condition score, dry matter as well as energy intakes during early lactation. It is well known that GH is very important for milk production in dairy cattle. Early studies suggested that insulin-hypoglycemia stimulated GH in sheep (Trenkle, 1971; Hertelendy and Kipnis, 1973) and 0.1 U/kg of insulin reliably results in hypoglycemia and GH secretion in humans (Jaffe *et al*., 1996). Also, Jaffe *et al*. (1999) conclusively demonstrate that insulin-hypoglycemia is a stimulus for GH secretion in sheep.

On the other hand, Carlson *et al*. (2007b) demonstrated that milk yield was lower for high carnitine (100 g/d) cows throughout the first 6 wk of lactation but milk yield did not differ among the control, low carnitine (6 g/d), and medium carnitine (50 g/d) treatments at any point. This was attributed to that demonstrated high dose dietary carnitine supplementation decreased liver triacylglycerol (TG) accumulation during the periparturient period as a result of enhanced capacity for hepatic long-chain fatty acids (LCFA) *β*-oxidation. However, La Count *et al*. (1995 and 1996a) indicated that milk production and energy balance were not influenced by post-ruminal infusion of carnitine to cows fed *ad. libitum*. Moreover, earlier reports observed that i.m. injections of cows with 0.2 to 0.6 IU (Kronfeld *et al*., 1963) and 0.35 IU (Schmidt, 1966) of rapid-release insulin/kg LBW depressed both milk and lactose yields. This depression of milk yield is due to hypoglycemia (Schmidt, 1966).

The confliction in the effect of L-carnitine or insulin on milk production may be attributed to treatment dose, or/and species or breed of treated animals. Also, the variation in the response to insulin was dependent on the animals being studied while they are fasting, and the magnitude of the GH rise is inversely related to the dose of insulin used (Jaffe *et al*., 1996).

Milk composition:

Results presented in Table (3) showed that milk composition including percentages of fat, protein, lactose, solid not fat and total solids in milk of cows were not affected significantly by treatment at different lactation months.

Table (3): Effect of treatment on milk composition of cows at different lactation months.

Milk component	Lactation month	Treatment (T)			
		T1(control)	T2 (INS)	T3 (LC)	T4 (INS+LC)
Fat	1	2.87±0.22	3.36±0.26	3.34±0.17	3.23±0.27
	2	3.53±0.25	3.11±0.27	3.25±0.17	3.43±0.26
	3	3.28±0.23	3.45±0.23	3.64±0.14	3.99±0.26
	4	3.47±0.12	3.88±0.20	3.66±0.08	4.06±0.26
Protein	1	2.18±0.15	2.77±0.37	2.58±0.17	2.13±0.19
	2	2.75±0.28	2.45±0.22	2.70±0.18	2.65±0.16
	3	2.58±0.17	2.56±0.19	2.77±0.17	2.72±0.18
	4	2.68±0.10	2.79±0.16	2.82±0.21	2.65±0.21
Lactose	1	4.98±0.14	4.90±0.47	4.65±0.20	4.30±0.39
	2	4.86±0.14	4.61±0.32	4.94±0.14	4.83±0.13
	3	4.98±0.12	4.95±0.10	5.07±0.10	4.94±0.09
	4	5.13±0.19	4.90±0.19	5.13±0.15	4.84±0.11
TS	1	10.72±0.41	11.71±1.01	11.23±0.32	10.19±0.81
	2	11.83±0.40	10.88±0.77	11.59±0.24	11.60±0.44
	3	11.54±0.33	11.65±0.38	12.18±0.20	12.35±0.45
	4	11.90±0.10	12.27±0.32	12.31±0.24	12.27±0.47
Solids not fat	1	7.85±0.25	8.35±0.82	7.89±0.33	6.96±0.62
	2	8.31±0.23	7.76±0.52	8.35±0.26	8.17±0.23
	3	8.26±0.18	8.20±0.22	8.54±0.18	8.36±0.19
	4	8.35±0.14	8.39±0.19	8.65±0.18	8.18±0.21

a and b:Means denoted within the same row with different superscripts are significantly different at P<0.05).

As affected by parity of cows, results in Table (4) showed that percentages of protein, solids not fat and total solids in milk of cows was affected by parity of cows during the 2nd month of lactation, being higher in multi- than in primi-parous cows. It was found that increased solids not fat and total solids in milk of multi-parous cows was mainly related to increasing protein percentage in their milk with insignificant differences in milk yield (Table 4).

The effect of interaction between treatment and parity was significant (P<0.05) only on lactose and total solids during the 2nd month of lactation. Such trend of differences in the effect of parity or its interaction during the 2nd month of lactation may suggest that most changes in milk composition occurred during this interval of milk production. These changes may be due to the interaction between parity of cows with type of treatment.

In accordance with the present results, Carlson *et al*. (2007b) found that protein, lactose and total solids percentages in milk were not affected by carnitine treatments. Also, Carlson *et al*. (2006) found that abomasal carnitine infusion (20 g/d) during short-term feed restriction did not affect milk fat percentage. Moreover, Darragh and Mouigha (1998) indicated that the milk composition, i.e., the concentrations of protein, fat and lactose in the milk of the sows, also is consistent. In general, milk composition was not influenced by post-ruminal infusion of carnitine to cows fed *ad. Libitum* (La Count *et al*., 1995, 1996a).

Table (4): Effect of parity on milk composition of cows at different lactation months.

Milk component	Lactation month	Parity (P)		Inter.
		Primi-parous cows	Multi-parous cows	T x P
Fat (%)	1	3.11±0.17	3.29±0.16	0.604
	2	3.26±0.17	3.40±0.17	0.307
	3	3.60±0.12	3.58±0.21	0.649
	4	3.81±0.09	3.72±0.17	0.889
Protein (%)	1	2.37±0.22	2.46±0.13	0.898
	2	2.37±0.11[b]	2.90±0.14[a]	0.421
	3	2.50±0.10	2.81±0.12	0.996
	4	2.60±0.09	2.87±0.13	0.907
Lactose (%)	1	4.85±0.23	4.56±0.23	0.325
	2	4.75±0.16	4.87±0.11	0.011**
	3	5.02±0.06	4.95±0.08	0.108
	4	5.05±0.06	4.95±0.08	0.441
Total solids (%)	1	11.01±0.54	10.91±0.46	0.858
	2	11.08±0.38[b]	11.87±0.28[a]	0.050*
	3	11.82±0.19	12.05±0.31	0.634
	4	12.16±0.13	12.21±0.28	0.780
Solids not fat (%)	1	7.91±0.42	7.62±0.38	0.733
	2	7.83±0.25[b]	8.47±0.18[a]	0.060
	3	8.22±0.11	8.47±0.15	0.662
	4	8.35±0.10	8.44±0.16	0.668

a and b: Means denoted within the same row with different superscripts are significantly different at P<0.05).

Reproductive performance:

Data in Table (5) showed that average interval from treatment to service estrus (ITE), or to conception (ITC) and consequently number of doses required to conception was the shortest in cows treated with LC (T3). However, cows in other treatment groups (T2 and T4) showed nearly similar values.

In addition, cows in T3 showed significantly (P<0.05) shorter postpartum first estrus (PPFEI) and service (PPFSI) intervals as compared to the control group, being 58.9 and 65.2 d, respectively. These intervals were 86.9 and 95.3 d, respectively, in the control group (T1). Moreover, cows in T3 showed significantly (P<0.05) high conception rate (CR, 78%) which was significantly (P<0.05) associated with the least number of service per conception (NSC, 1.33 services), lowest days open (DO, 81.4 d) and the shortest calving interval (CT, 368 d). On the other hand, cows in T2 and T4 showed acceptable results regarding NSC, DO and CI, being better than that in T1 (control). Yet, T4 showed significantly (P<0.05) the highest CR (90%) as compared to other treatment groups and the control one. Based on these results, all treatments resulted in improving postpartum reproductive performance of cows, being the best for those treated with LC.

Table (5): Effect of treatment on reproductive performance of cows during 120 day-postpartum.

Item	Treatment (T)			
	T1 (control)	T2(INS)	T3(LC)	T4 (INS+LC)
ITE (d)	-	64.3±9.38	48.7±6.70	60.3±9.48
NTC (n)	-	9.7±1.33	7.7±1.03	9.1±1.39
ITC (d)	-	66.2±10.44	62.1±9.87	78.6±6.51
PPFEI (d)	86.9±8.25^{b}	73.5±9.16^{ab}	58.9±6.62^{a}	51.2±8.77^{a}
PPFSI (d)	95.3±7.88^{b}	77.8±8.40^{ab}	65.2±6.31^{a}	74.7±8.55^{ab}
Conception rate (%)	44^{c}	60^{bc}	78^{ab}	90^{a}
NSC/cow	1.78±0.28	1.50±0.27	1.33±0.17	1.60±0.22
Days open	106.5±7.44^{b}	83.8±8.04^{a}	81.4±7.10^{a}	92.6±6.60^{ab}
Calving interval (d)	396±39.66	371±31.11	368±21.17	381±18.77

a and b:Means denoted within the same row with different superscripts are significantly different at P<0.05).
NTC: Number of treatments per conception. ITE: Interval from treatment to estrus.
ITC: Interval from treatment to conception. PPFEE: Postpartum first estrus interval.
PPFSI: Postpartum first service interval. DO: Days open. CI: Calving interval.

As affected by parity, values of ITE, NTC, ITC, PPFEI, PPFSI, DO and CI were significantly (P<0.05) lower, while NSC and CR was significantly greater in multi- than in primi-parous cows. The effect of interaction between treatment and parity on all reproductive traits was not significant (Table 6).

Previous studies reported reduced plasma total carnitine level in dairy cattle during lactation period (Roos *et al*., 1992 and Citil *et al*., 2003). It was suggested that discharge of carnitine along with milk secretion results in decreased levels of carnitine in dairy cows during lactation.

Table (6): Effect of parity on reproductive performance of cows during 120 day-postpartum.

Item	Parity (P)		Interaction
	Primi-parous cows	Multi-parous cows	T x P
ITE (d)	69.67±5.27^{a}	40.60±4.84^{b}	P<0.347
NTC (n)	11.11±0.70^{a}	6.35±0.72^{b}	P<0.273
ITC (d)	81.11±7.19^{a}	62.41±5.13^{b}	P<0.156
PPFEI (d)	77.8±7.94^{a}	44.4±5.87^{b}	P<0.143
PPFSI (d)	87.5±5.67^{a}	56.3±5.93^{b}	P<0.229
CR (%)	50^{b}	85^{a}	-
NSC/cow	1.17±0.09^{b}	1.90±0.18^{a}	P<0.139
Days open	98.00±6.61^{a}	78.24±5.64^{b}	P<0.176
Calving interval (d)	480±20.64^{a}	406±17.22^{b}	P<0.314

A and B: Means denoted within the same row with different superscripts are significantly different at P<0.05).
NTC: Number of treatments per conception. ITE: Interval from treatment to estrus.
ITC: Interval from treatment to conception. PPFEE: Postpartum first estrus interval.
PPFSI: Postpartum first service interval. DO: Days open. CI: Calving interval.

Therefore supplemental L-carnitine may help to improve lactation performance in ruminants. In agreement with the present results of cows in T3, the effect of L-carnitine on improving the fertility was observed by

Pirestani *et al*. (2011), who indicated that L-carnitine have beneficial effect on reducing calving interval to first estrus and to the first service, open days and number of services per pregnancy. Recent study of Dunning and Robker (2012) demonstrated that L-carnitine improved *in vitro* oocyte maturation and embryo growth. In addition, using the L-carnitine in a 40-day period were reduced open days, calving interval to first service (Carlos *et al.*, 2007). Dietary L-carnitine either increases the ovulation rate or reduces embryonic mortality in sows (Eder (2005). Noseir *et al*. (2003) reported that time intervals to first ovulation as well as first estrus significantly (P<0.05) decreased in buffalo cows receiving L-carnitine supplementation (41.5 and 62.2 d) compared to the controls (58.0 and 75.8 d), respectively. The interval to first service or conception (days open) significantly (P<0.01) shortened by L-carnitine supplementation, being 95.8 and 119.6 d in the control group compared with 74.3 and 81.6 d in L-carnitine supplemented group, respectively. Number of services per conception also decreased (P<0.01) in buffalo cows receiving L-carnitine (1.40 services) compared to the control (2.0 services).

L-carnitine plays an important role in the production of energy via mitochondrial β- oxidation in cells (De Vivo and Tein, 1990). Carnitine is shown to have important functions in some metabolic processes such as oxidation of long-chain fatty acids (Oven *et al*., 1996), regulation of ketosis (Mc Garry *et al*., 1975), support of the immune system (Famularo and De Simone, 1995) and enhancement of the antioxidant system (Arockia Rani and Panneerselvam, 2001; Marasli *et al*., 2005) and improvement of reproduction. Likewise, the improvements in reproductive performance of sows fed diets with carnitine may be reflective of elevated circulating leptin and other hormones and metabolites. Therefore, the beneficial responses to sow reproductive performance observed with LC (Musser *et al*., 1999; Eder *et al*., 2001). The effect of L-carnitine could be associated with stimulation of lipid metabolism through transfer of acyl groups across the mitochondrial membranes (Oven *et al*., 1996). Erfle *et al*. (1974) demonstrated that carnitine supplementation was able to lower free fatty acids and keton bodies in cows suffering from ketosis and NEFA in healthy cows. Kacar *et al*. (2010) reported that L-carnitine administrations increased glucose (the main source of energy) concentration in advanced pregnant Damascus goats with multiple pregnancies.

Progesterone profile:

Results presented in Table (7) showed insignificant effect of treatment on serum P4 concentration pre-treatment, one, two and three weeks post-treatment, and at 1st estrus and conception. The significant (P<0.05) effect of treatment appeared at pregnancy. Serum P4 concentration was significantly (P<0.05) higher in T4 than in T2 and T3, but did not differ from that in T1 (control). However, the differences in serum T4 between both T2 and T3 and T1 were not significant.

Table (7): Effect of treatment on progesterone concentration in blood serum of cows.

Item	Treatment (T)			
	T1(control)	T2 (INS)	T3 (LC)	T4 (INS+LC)
Pre-treatment (0 time)	0.901±0.29	0.824±0.19	1.04±0.21	0.842±0.19
One week post-treatment	1.581±0.57	1.784±0.79	6.613±5.06	0.677±0.11
2 weeks post-treatment	1.332±0.51	1.927±0.53	7.990±4.97	1.255±0.49
3 weeks post-treatment	1.577±0.90	2.011±0.67	3.593±1.31	1.454±0.43
At first estrus	0.463±0.06	0.566±0.08	0.431±0.04	0.624±0.16
At estrus of conception	0.407±0.16	0.630±0.16	0.620±0.15	0.502±0.10
At pregnancy	7.561±1.00[ab]	6.004±1.04[b]	7.172±0.78[b]	9.221±0.42[a]

a and b:Means denoted within the same row with different superscripts are significantly different at P<0.05).

As affected by parity, the differences in serum P4 concentration pre-treatment, one, two and three weeks post-treatment, and at 1st estrus between primi- and multi-parous cows were not significant. However, serum P4 concentration was significantly (P<0.05) higher in multi- than in primi-parous cows at conception and pregnancy (Table 8).

The observed values of P4 concentration pre- and 3 wk post-treatment were higher than 0.5 ng/ml, indicating the cyclicity of most cows in the experimental groups. Quintans *et al*. (2004) reported that buffalo cows were assumed to resume cyclicity when the serum progesterone level was 0.5 ng/ml.

Table (8): Effect of parity on progesterone concentration in blood serum of cows.

Item	Parity (P)		Inter.
	Primi-parous cows	Multi-parous cows	T × P
Pre-treatment	0.874±0.13	0.941±0.17	0.116
One week post-treatment	3.571±2.56	1.750±0.47	0.251
Two weeks post-treatment	1.743±0.50	4.501±2.54	0.610
Three weeks post-treatment	1.831±0.64	2.494±0.64	0.404
At first estrus	0.588±0.18	0.454±0.13	0.071
At estrus of conception	0.353±0.11[b]	0.659±0.08[a]	0.785
At pregnancy	9.072±0.43[b]	6.930±0.55[a]	0.086

a and b: Means denoted within the same row with different superscripts are significantly different at P<0.05).

Also, serum P4 concentration at 1st estrus or at estrus of conception of all groups in this study was around 0.5 ng/ml revealing incidence of estrus by monitoring P4 profile. Plasma progesterone concentrations are affected by energy balance of dairy cows (Vasconcelos *et al*., 2003). In this respect, Kendrick *et al*. (1999) found that cows in better energy balance (high energy) had greater intra-follicular IGF-I and plasma progesterone and tended to produce more oocytes graded as good. Also, several authors reported that insulin affected the CL diameter (Selvaraju *et al*., 2002; Sarath *et al*., 2008). In this line, P4 concentration in T2 and T3 was not affected by insulin or L-carnitine alone, which is contrary to the previous findings. However, the

combined treatment of both treatments may have marked effect on CL diameter and consequently increasing (P<0.05) P4 concentration in T4 as compared to T2 or T3 at pregnancy. This effect is similar to long chain fatty acid supplementation, which was able to increase serum P4 (El-Shahat and Abo-Elmaaty, 2010).

Progesterone promotes the establishment of a favorable uterine environment for survival of the developing embryo and is thus critical for successful pregnancy serum P4 before and after estrus is associated positively with fertility of cattle (Catchpole, 1991). This finding was associated with the highest CR (90%) of cows in T4 at pregnancy, which significantly (P<0.05) showed the highest P4 level at pregnancy. The progesterone concentration in pregnant does treated with insulin was highest on day 9 after mating, indicating the presence of a well developed and functional CL (Peixoto Pinheiro *et al*., 2012).

Conclusion

Generally, the insignificant effect of the interaction between treatment and parity of cows on most traits studied in this work indicated the possibility of using these treatments in lactating cows, regardless their parity. Based on the foregoing results of the current study, administration with an i.m injection of 40 IU insulin plus oral dose of 1 g LC/100 kg LBW during early postpartum period may consider successful treatment for improving milk yield and reproductive performance of lactating Friesian cows (primi- and multi-parous) under the experimental conditions.

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تأثير بعض العوامل الميتابوليزمية أثناء فترة بعد الولادة على إنتاج اللبن و الأداء التناسلى لأبقار الفريزيان فى الموسم الأول و المواسم المتعددة

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لتقيم تأثير بعض العوامل الميتابوليزمية مثل الأنسولين و L – كارنتين على إنتاج اللبن و الأداء التناسلى للأبقار فى الموسم الأول و المواسم المتعددة أثناء فترة ما بعد الولادة ، ٤٠ بقرة فريزيان (٢٠ فى الموسم الأول و ٢٠ فى المواسم المتعددة) قسمت إلى ٤ مجموعات متشابهة (١٠ حيوانات فى كل مجموعة ، ٥ حيوانات فى الموسم الأول P1 و ٥ حيوانات فى المواسم المتعددة P2).

المجموعة الأولى كنترول ، بينما المجموعة الثانية تم حقنها إسبوعيا ٤٠ وحدة دولية أنسولين/١٠٠ كجم من وزن الجسم الحى. المجموعة الثالثة تم تجريعها إسبوعيا ١جم L – كارنتين/١٠٠ كجم من وزن

الجسم الحى ، بينما فى المجموعة الرابعة تم حقن الحيوانات بالأنسولين بجرعة ٤٠ وحدة دولية بالإضافة إلى تجريعها ١ جم L – كارنتين/١٠٠ كجم من وزن الجسم الحى إسبوعيا. أجريت هذه المعاملات خلال ٧-١٠ أيام بعد الولادة حتى أول تلقيح. كل الحيوانات تم إبقاؤها تحت نفس الرعاية و نظام التغذية. و تم حلبها مرتين يوميا بواسطة المحلب الألى. تم تسجيل إنتاج اللبن إسبوعيا و تم تحليل تركيب اللبن شهريا لمدة ٤ شهور حليب. دخلت الحيوانات فى دورة شياع خلال ٤٠-٤٥ يوم بعد الولادة و لقحت صناعيا و تم تشخيص الحمل بعد ٤٥ يوم من التلقيح.تم أخذ عينات الدم فى ٠ ، ١ ، ٢ ، ٣ أسبوع من المعاملة ،تم إختبار الشياع و الحمل عن طريق قياس تركيز هرمون البروجستيرون فى سيرم الدم. تم قياس الصفات التناسلية المختلفة بعد الولادة و لمدة ٤ شهور.

يتضح من النتائج المتحصل عليها أن متوسط إنتاج اللبن الإسبوعى إرتفع (P<0.05) فى المجموعة الرابعة عن المجموعة الأولى ولم يكن هناك إختلاف بين المجموعة الثانية و الثالثة خلال شهر الحليب الثانى. توجد زيادة غير معنوية فى متوسط إنتاج اللبن الإسبوعى فى المجموعة الثانية و الثالثة بالمقارنة بالمجموعة الأولى. توجد زيادة معنوية فى متوسط إنتاج اللبن اليومى أو إنتاج اللبن الكلى (P<0.05) فى المجموعة الثانية و الثالثة و الرابعة ١٣ ، ١٤ ،١٥% بالمقارنة بالمجموعة الأولى على التوالى. لم يتأثر متوسط إنتاج اللبن اليومى و الإسبوعى و الكلى بعدد المواسم ، لم يتأثر تركيب اللبن بالمعاملة فى مختلف شهور الحليب. كانت نسبة البروتين و المواد الصلبة اللادهنية و المواد الصلبة الكلية فى اللبن خلال الشهر الثانى من الحليب مرتفعة (P<0.05) فى الحيوانات متعددة المواسم (P2) عن الحيوانات فى الموسم الأول (P1). متوسط الفترة من المعاملة حتى الشياع أو الإخصاب و عدد الجرعات المطلوبة حتى الإخصاب كانت أقل فى المجموعة الثالثة أما المجموعة الثانية و الرابعة كانت متشابهة. كانت المجموعة الثالثة أقل (P<0.05) فى الفترة من بعد الولادة حتى أول شياع و كذلك الفترة من الولادة حتى أول تلقيح من المجموعة الأولى (٥٨,٩ ، ٦٥,٢ ، ٨٦,٩ ، ٩٥,٣ يوم على الترتيب). كانت المجموعة الثالثة عالية (P<0.05) فى نسبة الإخصاب (٧٨%) و عدد التلقيحات اللازمة للإخصاب (١,٣٣) و كانت الأقل فى الأيام المفتوحة (٨١,٤) و كانت الأقصر فى الفترة بين ولادتين (٣٦٨ يوم) ، بينما المجموعة الرابعة كانت أعلى (P<0.05) فى نسبة الإخصاب (٩٠%) ، الفترة من المعاملة حتى الشياع ، و عدد المعاملات لكل إخصاب ، و الفترة من المعاملة حتى الإخصاب ، والفترة من الولادة حتى أول شياع ، و الفترة من الولادة حتى أول تلقيح ، و الفترة من الولادة حتى الإخصاب ، و كانت الفترة بين ولادتين أقل (P<0.05) ، بينما كانت عدد التلقيحات اللازمة للإخصاب و نسبة الإخصاب أفضل (P<0.05) فى المجموعة الثانية عن الأولى.

تركيز البروجستيرون فى الدم فى ٠ ، ١ ، ٢ ، ٣ أسبوع من المعاملة ، الشياع ، الشياع المخصب لم تتأثر بالمعاملات. كان تركيز البروجستيرون فى السيرم عند الحمل مرتفعا (P<0.05) فى المجموعة الرابعة عن المجموعة الثانية و الثالثة و لكن لا يوجد إختلاف عن المجموعة الأولى.كان تركيز البروجستيرون فى السيرم مرتفعا (P<0.05) فى المجموعة الثانية عن المجموعة الأولى فقط عند الشياع المخصب و الحمل.

الخلاصة أن المعاملة بالحقن بواسطة ٤٠ وحدة دولية أنسولين بالإضافة إلى التجريع ١ جم L-كارنتين/١٠٠كجم من وزن الجسم الحى أثناء فترة بعد الولادة مبكراً تعتبر معاملة ناجحة لتحسين إنتاج اللبن و الأداء التناسلى لأبقار الفريزيان الحلابة فى الموسم الأول و المواسم المتعددة تحت ظروف هذه التجربة.

الكلمات المفتاحية: الابقار، الموسم، الأنسولين، كارنتين، اللبن، التناسل و البروجستيرون

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