

EFFECT OF PROPYLENE GLYCOL ON REPRODUCTIVE PERFORMANCE IN POSTPARTUM PERIOD OF EGYPTIAN BUFFALOES

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ABSTRACT

Daily drenching of propylene glycol to buffalo cows in early lactation was used aiming at improving the reproductive performance through increasing plasma glucose and insulin. Twenty two buffalo cows were assigned to two groups (treated and control). Treated buffaloes were given a daily oral dose of propylene glycol from day 7 to day 40 postpartum. Plasma concentrations of glucose, insulin, and urea were measured once weekly during 2-9 weeks postpartum, and progesterone concentrations were measured twice weekly from 2 to 13 weeks postpartum. Propylene glycol increased plasma glucose concentrations in treatment group (63.06 ± 0.34 vs. 60.16 ± 0.67 mg/dl), and increased insignificantly insulin concentrations in treated buffaloes compared with control group. Plasma urea nitrogen (PUN) in the control group tended to be higher than that in the treatment group (37.21 ± 0.23 vs. 35.97 ± 0.16 mg/dl). The interval from calving to first ovulation in the treatment buffaloes were less than that in the control buffaloes. Days open period was less in treated group than that in the control (65.45 ± 7.5 vs. 81.81 ± 8.2 days). Conception rates at 50 days postpartum were 36% in the treated group and 18% in the control group. These results demonstrate that, administration of propylene glycol may improve ovarian function during early postpartum period in buffalo.

Keywords: Buffalo; Glucose; Insulin; Ovarian activity; Propylene glycol

INTRODUCTION

During the postpartum period, an increase in growth hormone and a decrease in insulin, thyroid hormones, and insulin-like growth factor (IGF-I) have been reported in cattle (Prandi *et al.*, 1992; Vega *et al.*, 1991) and in buffalo (Campanile *et al.*, 1997). This hormonal balance has negative impact on the reproductive performance.

Pate (1999) observed that changes in metabolic profile may negatively affect reproduction either directly on the ovary or indirectly via alteration of gonadotropin release. Insufficient energy intake results in poor reproductive performance, e.g. prolonged postpartum anestrus, low progesterone concentration, and low conception rate in dairy cattle (Butler and Smith, 1989; Britt, 1992) and in buffalo (Kanchev *et al.*, 1993).

Propylene glycol (PPG) is a gluconeogenic precursor used to treat ketosis in postpartum dairy cattle (Emery *et al.*, 1967; Grummer *et al.*, 1994; Christensen *et al.*, 1997). After oral administration, a portion of PPG is metabolized to propionate (Emery *et al.* 1964), but the majority of PPG escapes the rumen intact and is converted to glucose by the liver (Miller and Bazzano, 1995). Propionate is transported to the liver via the portal system, where it is transformed into glucose (Emery *et al.* 1967; Van Soest, 1994;

Moore and Ishler, 1997). Plasma concentrations of glucose, insulin and IGF-I are known to increase in response to PPG treatment (Studer et al., 1993; Grammar et al. 1994 Formigoni et al., 1996).

Follicle recruitment (Webb et al., 1999; Miyoshi et al., 2001; Spicer et al., 2002) as well as follicular growth and differentiation (Spicer and Echternkamp, 1995) are stimulated by insulin, and low levels of insulin delay the first ovulation, by acting on follicular development and / or LH secretion in the early postpartum (Miyoshi et al. 2001; Butler and Smith, 1989).

While IGF-I stimulates steroidogenesis, folliculogenesis, and ovulation (Yoshimura, 1998 and Lucy, 2000). Together, insulin and IGF-I affect ovarian function and early embryo development, on the contrary, with low levels of both IGF-I and insulin, the follicle does not produce adequate levels of estradiol or grow to a size able to trigger the LH surge and ovulation(Beam and Butler, 1999). Maintenance of progesterone synthesis requires insulin, which facilitates lipoprotein utilization in luteal cells (ul-Haq, 1992; Poff et al. 1998), in parallel, IGF-I stimulates CL growth and steroidogenesis (Alvarez et al. 2000). Therefore, progesterone could increase in response to increased plasma levels of insulin and IGF-I, whose concentrations in turn would be higher after a PPG drenching.

This work was planned to study the effect of treatment with propylene glycol on reproductive performance of Egyptian buffaloes in relation to some metabolic profile.

MATERIALS AND METHODS

1- Animals and treatments

A total of 22 buffalo cows, calved between October 2003 and March 2004, were used in this study, which extended for 90 days postpartum. They were divided to treated (G1) and control (G2) groups. Buffaloes of G1 were given an daily oral dose 450 ml. of propylene glycol (El Nasr Pharmaceuticals Co.) after feeding from day 7 to day 40 postpartum.

Cows were loosely housed in semi-shed open yards. Drinking water was made available all the time, animals were fed once daily and milked at 07.30 and 16.00 h. During the first 90 days of lactation, the average of milk production was 688 ±78 kg ; 671± 69 kg for the G2 and the G1, respectively.

Both groups were fed a concentrate mixture, barseem (*Trifolium alexandrium*) and rice straw during the period from December to the end of May, while during the rest of the year, the barseem was replaced with its hay or its silage. Table (1) shows the chemical composition of the ration ingredients.

Table 1: Chemical composition of the ration ingredients

Item	Concentrate mixture	Rice straw	Barseem hay
Moisture	12.6	7.5	10.0
Crude protein(CP)	11.9	3.5	11.9
Ether extract (EE)	3.9	----	----
Crude fiber (CF)	14.5	37.9	32.6

2- Blood sampling and assessment

Blood samples (10ml) were collected twice weekly, at 3-4 day intervals starting from day 14 through day 90 after calving, via jugular vein and drained in tubes containing EDTA (ethylene diamine tetra acetic acid). Samples were centrifuged at 3500 rpm for 10 min and the obtained plasma were stored at -20°C till hormonal and blood metabolites assay.

Insulin, glucose, and urea nitrogen (PUN) in plasma were measured once weekly from 2 to 9 weeks postpartum. Plasma insulin was assayed using a radioimmunoassay (RIA) kit (Coat-a-Count®; Diagnostic Products Co., Los Angeles, CA90045-5597, USA). Intra-assay and interassay coefficients were 5% and 4.9%, respectively. Plasma glucose and urea nitrogen (PUN) in plasma are determined by an enzymatic colorimetric method using commercial kit (Biodiagnostic). Progesterone concentrations were assessed twice weekly from the 2nd to the 13th week postpartum, by DSL-3900 ACTIVE® progesterone Coated – Tube Radioimmunoassay kit (Diagnostic System Laboratories, Inc. USA). The intra and inter assay variation coefficients were 4.8% and 9.2 %, respectively.

3- Reproductive parameters

The interval from calving to pregnancy (days open period) was calculated based on pregnancy diagnosis by rectal palpation. Conception rates at 50 days and at 100 days postpartum were calculated based on P₄ profile, palpation, and calving dates of the experimental groups. Days to first ovulation was assessed based on progesterone profile. Also, first progesterone peak during the first cycle postpartum was calculated based on progesterone profile.

On the basis of luteinic activity, the buffalo cows were classified into the following categories as described by Prandi *et al.* (1994): Acyclic buffaloes : (postpartum anoestrus), characterized by plasma progesterone concentrations being < 1 ng /ml; Cyclic buffaloes with normal cycles : one or two samples with progesterone < 1 ng /ml, followed by one sample with progesterone ≥ 1.2 ng/ml, followed by at least three samples with progesterone ≥ 2.0ng/ml, followed by one sample with progesterone < 1 ng/ml; short cycle : one or two samples with progesterone < 1 ng/ml, followed by one sample with progesterone ≥ 1.2 ng/ml, followed by two samples with progesterone ≥ 2 ng/ml, followed by one sample with progesterone < 1 ng/ml followed by a normal cycle; Pregnant buffaloes : with diagnosis performed by rectal palpation of buffaloes; buffaloes with alterations of the ovarian cycle (progesterone > 1.2 ng/ml in a single sample).

4- Statistical analysis

Data were analyzed using SAS (1999), the general linear models procedure GLM was used to calculate the analysis of variance. Duncan's multiple range test was used to calculate means separation for the studied variables. L S means was used also with T-test to determine the significant between studied variables. The nested model was used to analyze the repeated measurement data. The proposed model used was:

$$Y_{ijkl} = \mu + T_i + W_j + AN_k + AN_k(T)_i + (TW)_{ij} + \varepsilon_{ijkl}$$

Where,

Y is the vector of observation;

μ the overall means;

T_i the effect of i^{th} treatment, $i=1$ and 2 ;

W_j the effect of j^{th} week, $j=2,3,4,\dots,9$;

AN_k the effect of k^{th} animal, $k= 1,2,3,\dots, 15$;

$AN_k(T)_i$; the effect of the k^{th} animal within i^{th} treatment;

$(TW)_{ij}$ the interaction between i^{th} treatment and j^{th} week; and

ε_{ijkl} the effect of random error associated with the l^{th} individual assumed normally distributed with $(0, I\sigma^2_e)$.

RESULTS AND DISCUSSION

I-Metabolic profile:

Propylene glycol drenching increased ($P<0.05$) plasma concentrations of glucose during the period of treatment from 2 to 6 weeks postpartum, but afterward, the difference was not significant (Fig. 1).

Plasma insulin concentration in both groups was not significantly differed during the experimental period (Fig.2). These results are in agreement with Formigoni *et al.*,(1996). In contrast, other researchers have reported increased plasma glucose and insulin concentrations by 90 min of PPG administration (Christensen *et al.*, 1997; Miyoshi *et al.*, 2001).

Other authors observed similar plasma acute modifications on insulin, IGF-I and glucose levels in animals receiving oral doses of PPG as Grummer *et al.*(1994) and Studer *et al.* (1993).

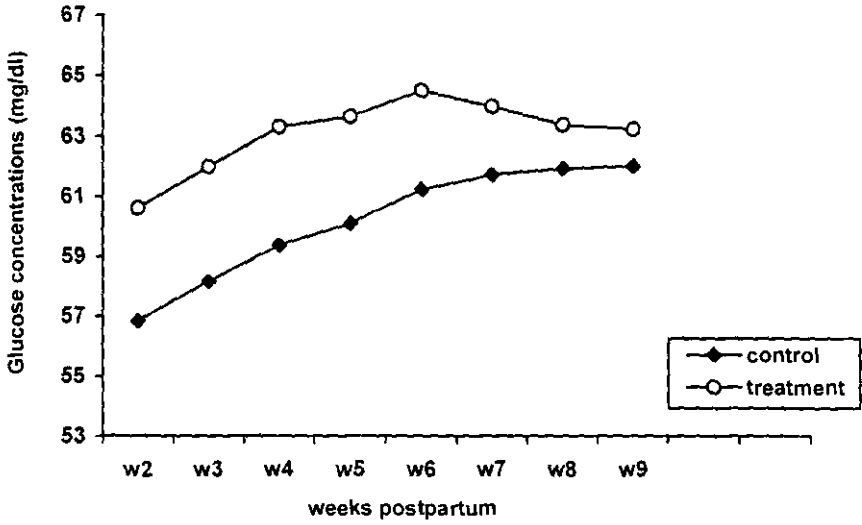


Fig.1 Plasma glucose concentrations for control and treated groups

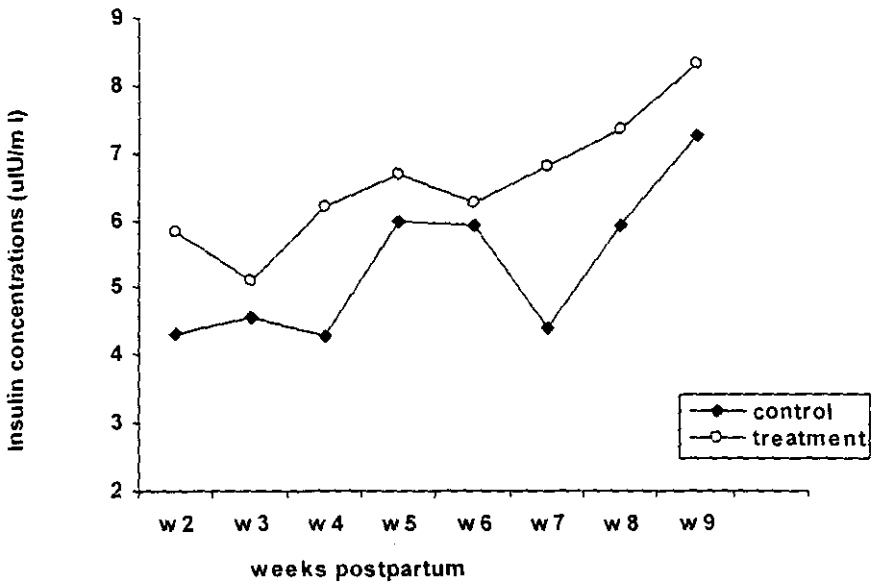


Fig.2 Plasma concentrations of insulin for control and treatment groups

According to the above reports, blood sampling within this study was planned to record non-acute effects of PPG administration, as it was hypothesized that the studied reproductive parameters would probably more dependent on sustained than short-term effects. Therefore, it would not expected to measure the acute surge in insulin and glucose concentrations reported in previous studies.

Less potential for a sustainable effect on insulin and glucose concentrations after cessation of the treatment (week 7-9) was expected (Table 2).

Plasma urea concentrations tended to be higher, however insignificant in G2 compared with G1 during the first 9 weeks of lactation (Fig. 3) and Table (2).

Formigoni *et al.* (1996) observed a similar trend in milk urea, as there is a strong correlation between urea content in blood and milk owing to the ability of urea to diffuse freely across the mammary tissue. Blood and milk urea content is known to be an indicator of nutritional status (Ropstad *et al.*, 1989) in the cow. Thus, the low blood urea levels observed in the treatment group provide further evidence that nutritional status was better for treatment animals than for control animals.

Table 2 : Means and standard errors of concentrations of plasma metabolites for control and treated buffaloes

Item	Treatment(G1)	Control(G2)
Glucose, mg/dl		
2-6 weeks	62.80 ± 0.8 ^a	59.13 ± 0.9 ^b
7-9 weeks	63.50 ± 0.9 ^a	61.87 ± 1.0 ^a
Plasma urea, mg/dl		
2-6 weeks	35.70 ± 0.5 ^a	37.07 ± 0.6 ^a
7-9 weeks	36.43 ± 0.7 ^a	37.49 ± 0.7 ^a
Insulin, µIU/ml		
2-6 weeks	6.01 ± 0.5 ^a	5.08 ± 0.5 ^a
7-9 weeks	7.50 ± 0.6 ^a	6.09 ± 0.6 ^a

Different letters in superscript in the same row express significant differences:(a,b)(P<0.05).

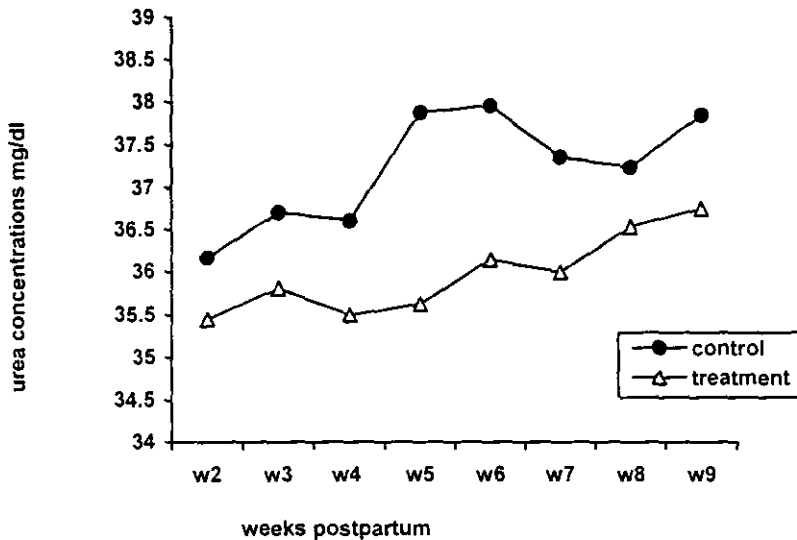


Fig. 3 Plasma urea nitrogen concentrations for control and treatment groups

II- Reproductive performance:

The interval from calving to the first postpartum ovulation, as confirmed by plasma progesterone concentration was insignificantly lower in the G1 than in the G2(Table 3).

Table 3: Reproductive parameters

Item	Treatment (G1)	Control (G2)
Days to 1 st ovulation (days)	18.6 ± 2.9	41.0 ± 12.3
First P ₄ peak (ng/ml)	5.32 ± 1.6	4.46 ± 1.1
Days open period(days)**	65.45 ± 7.5(n=11)	81.81 ± 8.2(n=11)
Conception rates at:		
50 days postpartum	4/11 (36%)	2/11 (18%)
100 days postpartum	7/11 (64%)	7/11 (64%)
130 days postpartum	--- ---	2/11 (18%)
Overall conception rate	11/11 (100%)	11/11 (100%)

**data based on P₄ assay (n = 5 / treatment group)

**data based on pregnancy diagnosis by palpation

The propylene glycol treated group showed higher P₄ levels than untreated group, this elevation of plasma P₄ level in the first cycle is consistent with the results observed by Miyoshi *et al.*(2001) in dairy cows.

Treatment with propylene glycol reduced the interval from calving to the first ovulation and to conception (Table 3), which coincidence with the findings of Campanile, (1993) in normally cyclic multiparous buffaloes (66.2 days). Treatment with PPG enhanced conception rates at 50 days postpartum. Although, the conception rates at 50 days postpartum were 36% in treated group versus 18% in control group, but it were the same (64%) in both groups at 100 days postpartum (Fig. 4).

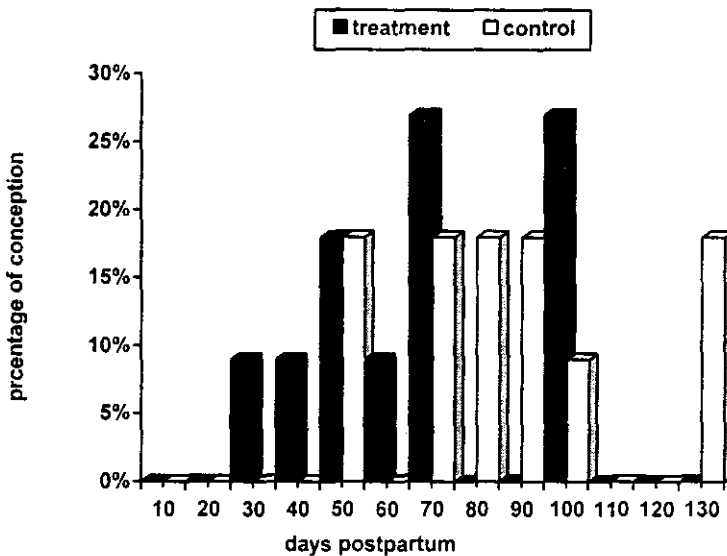


Fig. 4 Conception rates for control and treated groups

Concolusion

Oral administration of propylene glycol increased plasma concentrations of glucose and insulin, and lowered plasma urea nitrogen. Days to first ovulation and days open were decreased by treatment. Also, propylene glycol treatment increased plasma levels of progesterone. The results from this study indicate that administration of propylene glycol may improve ovarian function of buffaloes in early postpartum period, however, reproductive differences obtained failed to reach statistical significance.

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تأثير البريولين جليكول على الأداء التناسلي في الجاموس في فترة ما بعد الولادة
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أجريت التجربه بإفراض ان التجريع اليومي للجاموس بالبريولين جليكول في بداية موسم الحليب
سوف يؤدي إلى زيادة تركيزات البلازما من الجلوكوز و الإنسولين وبالتالي يحدث تحسين للخصوبه في
فترة ما بعد الولادة.

استخدم عدد ٢٢ جاموسه بعد الولاده ، تم توزيعها عشوائياً في مجموعتي المعامله و المقارنه. تم
التجريع اليومي لمجموعه المعامله بالبريولين جليكول في الفتره من اليوم ٧ و حتى اليوم ٤٠ بعد الولاده، تم
تقدير تركيزات البلازما للجلوكوز و الإنسولين و اليوريا مره أسبوعياً خلال الفتره من الاسبوع ٢ و حتى
الاسبوع ٩ بعد الولاده، و تم تقدير تركيزات البروجستيرون مرتين أسبوعياً من الاسبوع ٢ و حتى
الاسبوع ١٣ بعد الولاده.

أدت المعامله بالبريولين جليكول إلى زيادة تركيز الجلوكوز في البلازما ($٠,٣٤ \pm ٦٣,٠٦$
مقابل $٠,٦٧ \pm ٦٠,١٦$ ملجم / ديسلتر) كما زاد من تركيز الإنسولين زياده بسيطه عن مجموعه المقارنه ،
بينما قلت تركيزات البلازما من اليوريا في حيوانات المعامله عنها في حيوانات المقارنه ($٠,١٦ \pm$
 $٣٥,٩٧$ مقابل $٣٧,٢١ \pm ٧,٢٣$ ملجم/ديسلتر).

كانت الفتره منذ الولاده و حتى حدوث التبويض الأول أقل في مجموعه المعامله عنها في مجموعه
المقارنه ، و كان طول فتره الأيام المفتوحه أقل في مجموعه المعامله عنه في مجموعه المقارنه ($٧,٥ \pm$
 $٦٥,٤٥$ مقابل $٨,٢ \pm ٨١,٨١$ يوماً) . كانت معدلات الإخصاب عند ٥٠ يوماً بعد الولاده ٣٦% في
الحيوانات المعامله مقابل ١٨% في الحيوانات المقارنه . هذه النتائج توضح أن المعامله بالبريولين
جليكول ربما أدت إلى تحسين الأداء التناسلي خلال الفتره المبكره بعد الولاده في الجاموس.