Effects of eCG in Milking Dairy Cows with High Levels of BUN and Synchronized with Ovsynch Protocol

F. Karami1, A.H. Fallah Rad1*, M. Danesh Mesgaran2, M. Azizzadeh1 and M. Heidarpour1

1Department of Clinical Sciences, School of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.
2Department of Animal Science, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran.

http://dx.doi.org/10.13005/bbra/2096

(Received: 15 April 2016; accepted: 13 May 2016)

Previous research indicates that high blood urea nitrogen (BUN) concentrations are associated with decreased fertility in lactating dairy cows. This experiment was done to evaluate the effects of equine chorionic gonadotropin (eCG) administration (with or without eCG) on fertility of lactating dairy cows with different levels of BUN subjected to fixed-time artificial insemination (FTAI). Ovulation was synchronized in all cows using Presynch-Ovsynch program and start 37 days post parturition (pp). Blood samples were collected on days 50-55 pp to assess BUN and cows were classified as with high urea when BUN was 20< ng/mL (n=64); otherwise, they were classified as cows with optimal urea when BUN was >10 and <15 (n=62). Within each group, cows were assigned randomly to receive either 600 IU eCG concurrent with PGF2a treatment of the Ovsynch protocol (treatment cows) or with no further treatment (control cows). Blood samples were collected on Days 0 and 5 after FTAI to evaluate the effect of eCG administration on synchronization and plasma concentration of P4. Pregnancy diagnoses were performed by ultrasound. No treatment effects were detected for Pregnancy per AI at Days 30. However, P/AI at Days 30 was not affected by treatment with eCG but P/AI at 60 days post-AI in cow with high BUN levels in control group was less and differed significantly than treatment group with optimal BUN (OR=4.722; P=0.016). This differ significant, P/AI at 60 days post-AI no observed in cow with high BUN that treatment with eCG. P4 concentrations on Days 5 differed significantly between treatments and control group in both levels of BUN concentration. Inclusion of eCG in ovsynch protocol increased progesteron concentrations in early time of luteal phase after AI. The use of eCG in ovsynch protocol will improve reproductive efficiency in dairy cows with high levels of BUN.

Key words: Milking Dairy Cows, Synchronized, Ovsynch Protocol.

Increased genetic potential for milk production has been associated with a decline in fertility of lactating dairy cows. Strategies to meet the nutritional requirements of high producing cows has been necessarily changed in conjunction with genetic gains. High protein diets (17 to 19% CP) support and stimulate high milk production in early lactation24, 30, 46.

A consequence of high dietary crude protein is elevated plasma urea nitrogen (BUN) concentrations which have been associated with decreased fertility in dairy cows9,12,17. Reduced conception rates occur following sustained BUN greater than 19 mg/dL, or milk urea nitrogen (MUN) greater than 15.4 mg/dL9,17,45.

Excess protein, especially rumen degradable proteins; RDP, increases ammonia concentrations in the rumen, where it may become a nitrogen source for microbial protein. Ammonia
is toxic to animal tissues and when the circulating concentrations increase it is detoxified in the liver to urea, which is less toxic. Urea is a small, water-soluble molecule that permeates all cells and tissues in the body, and passes easily between blood and other tissues.

Three general mechanisms have been proposed to describe how excess dietary protein may negatively influence fertility: 1) Nitrogen byproducts may alter the uterine pH and mineral balance, 2) Nitrogen byproducts or efficiency of energy utilization may alter gonadotropin (P4) secretion, 3) Nitrogen byproducts may impair sperm, ova, or early embryo survival. These effects may occur alone, simultaneously, or synergistically.

Higher plasma P4 levels ameliorate some of the adverse effects of BUN on fertility after AI in dairy cows. Garcia-Bojalil et al. (1998) showed a negative effect of RDP on plasma P4, which could be restored by the inclusion of fat, resulting in an increased pregnancy rate with fat supplementation.

There have been numerous studies evaluating effects of P4 supplementation on fertility in cattle with the earliest experiments conducted in the 1950s. During the last 60 years, there have been numerous methods to increase P4 including: treatment with exogenous P4 (injectable P4; or P4 releasing intra-vaginal devices, PRID; or CIDR) or by treatments attempting to ovulate a follicle and produce an accessory CL using human chorionic gonadotropin (hCG) or gonadotropin releasing hormone (GnRH).

In non-equine species, eCG shows high LH- and FSH-like activities and has a high affinity for both FSH and LH receptors in the ovaries. On the granulosa and theca cells of the follicle, eCG has long-lasting LH- and FSH-like effects that stimulate oestradiol (E2) and P4 secretion. Thus, eCG administration in dairy cattle results in fewer atretic follicles, the recruitment of more small follicles showing an elevated growth rate, the sustained growth of medium and large follicles and improved development of the dominant and pre-ovulatory follicles. Consequently, the quality of the ensuing CL is improved, and thereby P4 secretion increased. Because of the LH- and FSH stimulating effects of eCG on follicular development, the possibility of improving conception rate by including eCG in synchronization and fixed time artificial insemination (FTAI) protocols has been evaluated. The positive effects of eCG are clearly detectable in cows in anoestrus or under seasonal heat stress conditions even improving fertility over spontaneous oestrous or in cows with lower (<2.75) BCS. Utilization of eCG in a synchronization protocol has also a positive effect on P4 plasma levels in the ensuing oestrous cycle.

However, there has been no investigation has examined the effect of use of eCG in dairy cattle with high levels of blood urea nitrogen and subsequent reproductive performance. This study was designed to determine whether addition of eCG in ovsynch protocols, improves reproductive performance and prevents fertility to decline in dairy cows with high levels of BUN and MUN.

MATERIALS AND METHODS:

Cow management

The present study was conducted in a commercial dairy herd with 4000 dairy cows located in the Isfahan Province, Iran. Selection of the herd was based on the availability of facilities to conduct the study. Briefly, cows were housed in free stall barns and milked three times daily. The rolling average of 305 days milk production was 11,500Kg. The voluntary waiting period was 45 days for multiparous cows. Cows were fed twice a day, with a TMR including corn silage, alfalfa hay, corn meal, barley, and a protein and mineral supplement.

Ovulation synchronization and treatments

Cows that had no degree of dystocia, retained placenta, abnormal uterine or vaginal discharge defined as purulent or mucopurulent discharge, were eligible for the experiment. Cows having a history of fever, mastitis, laminitis or any clinical disease after calving and received ecbolic drugs or hormones after calving were not included in the study.

Ovulation was synchronized in all cows using Presynch-Ovsynch program consisting of two consecutive treatments of PGF2α (2 mL IM injection of Estroplan, Parnell Technologies Pty LTD, Australia) on days 37 and 51 pp DIM, followed
by the traditional Ovsynch protocol (Fig. 1).

Ovsynch protocol consisted of two consecutive injections of 100 mg Gonadorelin IM (2 mL IM injection of Vetaroline, Aburaihan pharmaceutical company, Iran) on days 62 pp [GnRH 1] and 9.5 days later [GnRH 2]). Meanwhile, an IM injection of 2 mL PGF2a was administered on day 69 pp (2.5 days before GnRH-2 injection), with FTAI at 18 to 24 hours afterward (Fig. 1).

Blood samples were collected on days 50-55 to assess blood urea nitrogen concentrations. On the basis of serum BUN concentrations, cows were classified as with high urea when BUN was 20< ng/mL (n=64); otherwise, they were classified as cows with optimal urea when BUN was >10 and <15 (n=62).

Within each group, cows were assigned randomly to receive either 600 IU eCG (GONASER 5000, HIPRA, Spain) concurrent with PGF2a on day 69 pp (treatment cows) or with no further treatment (control cows). All cows were inseminated 72 hours after PGF2a (18 to 24 hours after GnRH-2).

Pregnancy was initially diagnosed on day 30 days post-AI and confirmed on day 60 days post-AI by trans-rectal ultrasonography (7.5 MHz linear-array transducer; BCF; Easi-Scan, Scotland, UK). A positive pregnancy diagnosis required the presence of anechoic uterine fluid and a large CL or with the presence of a viable embryo. Pregnancy loss was determined between the two pregnancy diagnoses.

Additional blood samples were collected on 0(TAI) and day 5 later to assess serum P4 and E2 concentrations. Response to Ovsynch protocol was determined on the basis of plasma concentrations on day 0. Cows with less than 1.0 ng/mL on day 0(TAI) were considered to have responded to the synchronization protocol.

After collection, blood samples were immediately cooled on ice before being centrifuged at 1500g for 20 minutes. Serum was separated and stored at -20° C until analysis day to determine P4 and E2 concentrations by ELISA (DRG ELISA Diagnostics Kit, GmbH, Germany).

**Statistical analysis**

Effect of treatment on chance of pregnancy was evaluated with multivariable logistic regression model. The model included treatment, parity and amount of fat, protein, somatic cell count and yield of milk in last record before insemination. Non-parametric Kruskal-Wallis test at p<0.05 was used to find out whether the level of P4 on the day of insemination and five days after it and E2 of the experimental groups was different significantly. Pair-wise comparison was conducted by the Mann-Whitney U-test. Since this was multiple testing of the data, the significance level was adjusted using Bonferroni test. The four groups were compared and therefore the significance level was calculated as 0.05 divided by 6; (p<0.0083).

**RESULTS**

In this study, blood samples were taken from 520 cow and analysed for BUN. Among these samples, 76 cows had BUN above 20 ng/mL and 245 cows had BUN above 10 and below 15 ng/mL and were selected for comparison with high BUN levels. These cows were assigned randomly to receive either eCG, or no treatment Effects of treatment on the risk of pregnancy was evaluated with multivariable logistic regression model. The model included treatment, parity and amount of fat, protein, somatic cell count and milk yield in the last record before insemination. Pregnancy per AI at 30 and 60 days post-AI, and subsequent pregnancy loss between diagnoses, are summarized in Table 1. No treatment effects were detected for pregnancy per AI on day 30 (Table 2). However, pregnancy per AI on day 30 was not affected by treatment with eCG but P/AI at 60 days post-AI in cows of the control group with high BUN levels was less and significantly different than treatment group with optimal BUN (OR=4.722; P=0.016) group (Table 3). Significant difference of P/AI at day 60 post-AI was not observed in cows with high BUN treated with eCG (P=0.246).

P4 and E2 concentrations are shown in (Table 4). P4 concentrations on days 5 were significantly different between treatments and control group in both levels of BUN concentrations. Treatment with eCG did not alter E2 concentrations when compared to the control cows.
Table 1. Effects of eCG on synchronized estrous, P/AI, Pregnancy loss, and Twin pregnancy dairy cows

<table>
<thead>
<tr>
<th>Levels</th>
<th>Group</th>
<th>No. of Cows</th>
<th>synchronized estrous No. (%)</th>
<th>P/AI at day 33, N (%)</th>
<th>P/AI at day 61, N (%)</th>
<th>Pregnancy loss from pregnancy d 23 to 53 after AI (N)</th>
<th>Pregnancy Twin (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>optimal BUN</td>
<td>Control*</td>
<td>32</td>
<td>31(96.6%)</td>
<td>13(41.9%)</td>
<td>13(41.9%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>eCG*</td>
<td>30</td>
<td>30(100%)</td>
<td>13(43.3)</td>
<td>13(43.3%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>High BUN</td>
<td>Control*</td>
<td>32</td>
<td>32(100%)</td>
<td>8(25%)</td>
<td>5(15.6%)</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>eCG*</td>
<td>31</td>
<td>30(96.7%)</td>
<td>11(36.7)</td>
<td>9(30%)</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

*Treatments: All cows were submitted to the same ovulation-synchronization protocol (GnRH on day 0, PGF2a on day 7, and GnRH on day 9). Cows in the eCG group received an injection of 600IU of eCG on day 7, whereas control cows received no further treatment.

Table 2. Odds ratios and variables included in final logistic regression model for conception rate at 30 days post AI, after treatment

<table>
<thead>
<tr>
<th>Variables in equation</th>
<th>B</th>
<th>SE</th>
<th>Wald test</th>
<th>df</th>
<th>Sig.</th>
<th>Exp. (B)</th>
<th>95% C.I. for Exp. (B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>optimal BUN</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>lower</td>
</tr>
<tr>
<td>Ovsynch</td>
<td>-0.086</td>
<td>0.548</td>
<td>0.024</td>
<td>1</td>
<td>0.876</td>
<td>0.918</td>
<td>0.314</td>
</tr>
<tr>
<td>High BUN</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>lower</td>
</tr>
<tr>
<td>Ovsynch</td>
<td>-0.324</td>
<td>0.544</td>
<td>0.355</td>
<td>1</td>
<td>0.551</td>
<td>0.723</td>
<td>0.249</td>
</tr>
<tr>
<td></td>
<td>eCG</td>
<td>-0.885</td>
<td>0.585</td>
<td>2.294</td>
<td>1</td>
<td>0.130</td>
<td>0.413</td>
</tr>
<tr>
<td>Parity</td>
<td>0.221</td>
<td>0.208</td>
<td>1.135</td>
<td>1</td>
<td>0.287</td>
<td>1.247</td>
<td>0.831</td>
</tr>
<tr>
<td>Milk</td>
<td>-0.019</td>
<td>0.024</td>
<td>0.625</td>
<td>1</td>
<td>0.429</td>
<td>0.981</td>
<td>0.936</td>
</tr>
<tr>
<td>Fat</td>
<td>-0.460</td>
<td>0.311</td>
<td>2.193</td>
<td>1</td>
<td>0.139</td>
<td>0.631</td>
<td>0.343</td>
</tr>
<tr>
<td>Protein</td>
<td>0.074</td>
<td>0.707</td>
<td>0.011</td>
<td>1</td>
<td>0.917</td>
<td>1.077</td>
<td>0.269</td>
</tr>
<tr>
<td>SCC</td>
<td>0.000</td>
<td>0.001</td>
<td>0.104</td>
<td>1</td>
<td>0.747</td>
<td>1.000</td>
<td>0.998</td>
</tr>
<tr>
<td>Constant</td>
<td>1.094</td>
<td>2.617</td>
<td>0.175</td>
<td>1</td>
<td>0.675</td>
<td>2.987</td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Odds ratios and variables included in final logistic regression model for conception rate at 60 days post AI, after treatment

<table>
<thead>
<tr>
<th>Variables in equation</th>
<th>B</th>
<th>SE</th>
<th>Wald test</th>
<th>df</th>
<th>Sig.</th>
<th>Exp. (B)</th>
<th>95% C.I. for Exp. (B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>optimal BUN</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>lower</td>
</tr>
<tr>
<td>Ovsynch</td>
<td>-0.122</td>
<td>0.548</td>
<td>0.050</td>
<td>1</td>
<td>0.823</td>
<td>0.885</td>
<td>0.302</td>
</tr>
<tr>
<td>High BUN</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>lower</td>
</tr>
<tr>
<td>Ovsynch</td>
<td>-0.646</td>
<td>0.557</td>
<td>1.344</td>
<td>1</td>
<td>0.246</td>
<td>0.524</td>
<td>0.176</td>
</tr>
<tr>
<td></td>
<td>eCG</td>
<td>-1.522</td>
<td>0.646</td>
<td>5.773</td>
<td>1</td>
<td>0.016</td>
<td>0.212</td>
</tr>
<tr>
<td>Parity</td>
<td>0.160</td>
<td>0.213</td>
<td>0.568</td>
<td>1</td>
<td>0.451</td>
<td>1.174</td>
<td>0.774</td>
</tr>
<tr>
<td>Milk</td>
<td>0.004</td>
<td>0.026</td>
<td>0.026</td>
<td>1</td>
<td>0.873</td>
<td>1.004</td>
<td>0.953</td>
</tr>
<tr>
<td>Fat</td>
<td>-0.462</td>
<td>0.325</td>
<td>2.016</td>
<td>1</td>
<td>0.156</td>
<td>0.630</td>
<td>0.333</td>
</tr>
<tr>
<td>Protein</td>
<td>0.120</td>
<td>0.731</td>
<td>0.027</td>
<td>1</td>
<td>0.870</td>
<td>1.127</td>
<td>0.269</td>
</tr>
<tr>
<td>SCC</td>
<td>0.000</td>
<td>0.001</td>
<td>0.003</td>
<td>1</td>
<td>0.956</td>
<td>1.000</td>
<td>0.998</td>
</tr>
<tr>
<td>Constant</td>
<td>-0.068</td>
<td>2.730</td>
<td>0.001</td>
<td>1</td>
<td>0.980</td>
<td>0.934</td>
<td></td>
</tr>
</tbody>
</table>
Table 4. Effect of eCG on P4 and E2 concentrations on days 0 and 5

<table>
<thead>
<tr>
<th>groups</th>
<th>Levels</th>
<th>No. of Cows</th>
<th>day 0 P4 (ng/mL)</th>
<th>day 5 P4 (ng/mL)</th>
<th>day 0 E2 (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Median Q1 Q3</td>
<td>Median Q1 Q3</td>
<td>Median Q1 Q3</td>
</tr>
<tr>
<td>Control</td>
<td>optimal BUN</td>
<td>31</td>
<td>0.25* .15 .40</td>
<td>2.4* 2.00 4.45</td>
<td>3.6 2.85 4.40</td>
</tr>
<tr>
<td>Control</td>
<td>High BUN</td>
<td>32</td>
<td>0.25* .14 .47</td>
<td>2.45* 1.90 3.65</td>
<td>3.65 2.80 4.85</td>
</tr>
<tr>
<td>eCG</td>
<td>optimal BUN</td>
<td>30</td>
<td>0.27* .15 .43</td>
<td>4.1* 2.70 5.10</td>
<td>3.4 2.70 4.40</td>
</tr>
<tr>
<td>eCG</td>
<td>High BUN</td>
<td>30</td>
<td>0.25* .13 .40</td>
<td>4.3* 3.20 5.90</td>
<td>3.6 2.50 4.70</td>
</tr>
</tbody>
</table>

Fig. 1. Diagram of the treatments for Presynch-Ovsynch timed AI program, with or without administration of 600IU eCG on day 69. Abbreviations: BUN, blood sampling and analysis of urea nitrogen; P4, blood sampling and analysis of serum P4 concentrations; US, ultrasonography; PD, pregnancy diagnosis by trans-rectal US; E2, blood sampling and analysis of serum E2 concentrations; TAI, timed AI

DISCUSSION

Our hypothesis that eCG treatment would moderate and can help to increase pregnancy/AI in cows with high levels of blood urea nitrogen (BUN) was not supported by the results of pregnancy test at 30 days post AI. Despite the numerical differences, detrimental effects of BUN on fertility was not significant, and probably, this was due to the sample size in each group. However, the pregnancy/AI at 60 days post AI was significantly different due to the detrimental effects of BUN on fertility in control group. Since, eCG treatment has increased P4 concentration within 5 days post AI, it is probable that slight and nonsignificant reduction in conception rate in high BUN animals was due to positive effects of eCG treatment.

In order to maximize milk production, many dairy cows are fed high protein diets. Urea is a product of protein breakdown and the peripheral urea nitrogen concentrations reflects protein metabolism. Urea diffuses into body fluids such as blood serum and milk and equilibrates in other parts of the body including, reproductive tissues. Both high and low circulating urea concentrations have been associated with reduced fertility in dairy cows, particularly increased calving to conception interval. However, these results are inconsistent within trials and no clear link (s) between fertility and protein metabolism had been established. Our results i.e. the difference in the pregnancy/AI in 30 days post service was not significant between cows with high and optimum BUN, but this parameter shows significant difference in 60 days post service pregnancy test, and reduced fertility in cows observed. This is probably due to the small sample size in each group. Cattle consuming diets that generate high levels of plasma ammonia cause high levels of ammonia in follicular fluids. Cultured oocytes with high concentrations of urea resulted in fewer oocytes successfully progressing to metaphase II. When oocytes were exposed to high levels of urea in vitro or to metabolism of high protein diets in vivo, the resulting embryos were less likely to develop to the blastocyst stage. In several studies that eCG was applied in certain times, similar to the
present study, it was reported that eCG either
increased\textsuperscript{47} the diameter of the preovulatory follicle
or, had no effect\textsuperscript{31,49}. Administration of eCG might
reduce variation in ovulation time range and could
increase quality of the zygote, thus, resulting in
improvement of fertility to timed-AI protocols\textsuperscript{13}. Among actively growing follicles, larger follicles
contained oocytes with greater nuclear maturation
and resulted in greater success by in-vitro
maturation (IVM) and in-vitro fertilization (IVF) in
several livestock species\textsuperscript{23, 28, 38}. Lamb et al. and
Vasconcelos et al. were the first to report increased
pregnancy rates in cows that had ovulated larger
follicles\textsuperscript{32, 56}. In the present study, eCG effects on
preovulatory follicle diameter and time of ovulation
was not measured but, this positive effect of eCG
might have affected our results.

The cervical mucus (CM) represents the
biological environment-matrix affecting sperm
survival and determines ability of the cow to
conceive. The crystallization pattern “arborisation”
of CM could be used to detect oestrus. The CM
during estrus shows a fern pattern of crystallization
showing the best time for AI\textsuperscript{25}. Toxic metabolites
such as urea can accumulate in CM as an indicator
of a cow’s metabolism intensity and can negatively
affect the survival and/or fertilization ability of
sperms\textsuperscript{3}. High concentrations of urea in CM can
cause decreased sperm motility and survival. And
in addition, decreases ferny-like patterns and
inhibits atypical and/or stellate structures. In
general, it causes reduction in CM quality\textsuperscript{4}. In the
estrus, under the influence of estrogen, there is an
increase in muco-protein secretions, sodium
chloride and water content of CM which alter the
pH of the CM before, during and after estrus,
Alkaline pH of CM is favorable for sperm motility\textsuperscript{43},
so, with increased levels of estrogen, probably CM
quality improves. The eCG treatment increased
dominant follicle diameter\textsuperscript{42}. Follicles of larger
diameters cause an increase in E2 concentrations\textsuperscript{42}. In our results, treatment with eCG failed to alter E2
concentrations as compared to the animals in both
control groups at the time of AI. Because serum E2
concentrations peak approximately 36 h before
ovulation\textsuperscript{44}, therefore, showing eCG effects on E2
concentrations needs to be repeat around the time
of AI.

P4 plays a key role in the reproductive
events of the cow\textsuperscript{1}, the effects of P4 in inducing
dramatic changes in the uterus, termed
progestogen changes, are essential to produce
an environment compatible with embryonic growth,
implantation and maintenance of pregnancy\textsuperscript{7}. High
dietary CP reduced plasma P4 concentrations in
lactating cows in three of four studies\textsuperscript{6,29,50,52}. Bovine endometrial cells in culture responded
directly to increasing urea concentrations with
alteration in pH gradient but responded most
notably with increased secretion of prostaglandin
F2a (PGF2a)\textsuperscript{21}. Increased uterine luminal PGF2a
interferes with embryonic development and survival
in dairy cows. High concentrations of circulating
P4 in the immediate post-conception period have
been associated with an advancement of
conceptus elongation, and is associated with
increase in interferon-t production\textsuperscript{7,35,36,54}. Higher
plasma P4 levels ameliorate some of the adverse
effects of BUN on fertility after AI in dairy cows\textsuperscript{11}.
Garcia-Bojalil et al. (1998) showed negative effects
of RDP on plasma P4, which could be restored by
the inclusion of fat in the diet, resulting in an
increase in pregnancy rate with fat
supplementation\textsuperscript{18}. The increased P4 have been
done by inducing formation of an accessory CL
with hCG or GnRH treatment. When hCG or GnRH
is administered on day 5 after AI, in general, there
is a formation of an accessory CL and increase in
P4 by day 9. It seems that day 9 is not early enough
in the cycle to induce uterine changes that are
needed to optimize fertility\textsuperscript{86}. Administration of
eCG before ovulation, also leads to augmentation
of plasma P4 levels during the oestrous cycle
subsequent to the treatment\textsuperscript{7,80,85}. Our results were
similar to those reports in which eCG was
administered concurrent with PGF2a and affected
p4 concentrations in dairy cattle. In our study,
increased p4 concentrations after eCG treatment
was significant in both groups. Probably,
enhancement of p4 concentrations in early luteal
phase was similar to Garcia-Bojalil et al. study which
ameliorated some of the adverse effects of BUN
on fertility after AI. and restriction of significant
difference between pregnancy/AI in with levels of
BUN.

CONCLUSION

In conclusion, high levels of BUN
decrease reproduction efficiency in dairy cows.
The use of eCG in ovsynch protocol increased progesteron concentrations in early time of luteal phase after AI. Higher plasma P4 levels ameliorate some of the adverse effects of BUN on fertility after AI in dairy cows.

ACKNOWLEDGEMENTS

This research was financially supported by Ferdowsi University of Mashhad which is greatly appreciated. Special thanks to FKA dairy farm management in Esfahan allowing us to run the experiment in that farm. We express our kind appreciation to the personnel of the farm particularly Dr Mehdi Safahani-Langroudi for his cooperation and advice during the experiment.

REFERENCES


25. Hafez, B., Hafez, E.S.E., Reproduction in Farm Animals. Maryland, USA, 2000; 495.


44. Perry GA, Smith MF, Lucy MC, Green JA, Parks TE, MacNeil MD, et al. Relationship between follicle size at insemination and


