BIOSCIENCES BIOTECHNOLOGY RESEARCH ASIA, June 2016. Vol. **13**(2), 769-777

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

F. Karami[1], A.H. Fallah Rad[1]*, M. Danesh Mesgaran[2], M. Azizzadeh[1] and M. Heidarpour[1]

[1]Department of Clinical Sciences, School of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.
[2]Department 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 synchornization 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 progestron 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[8]. 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 lactation[24, 30, 46].

* To whom all correspondence should be addressed.

A consequence of high dietary crude protein is elevated plasma urea nitrogen (BUN) concentrations which have been associated with decreased fertility in dairy cows[9,12,17]. Reduced conception rates occur following sustained BUN greater than 19 mg/dL or milk urea nitrogen (MUN) greater than 15.4 mg/dL[9,17,45].

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[10].

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 and (or) progesterone (P4) secretion, 3) Nitrogen byproducts may impair sperm, ova, or early embryo survival. These effects may occur alone, simultaneously, or synergistically[5].

Higher plasma P4 levels ameliorate some of the adverse effects of BUN on fertility after AI in dairy cows[11]. 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[18].

There have been numerous studies evaluating effects of P4 supplementation on fertility in cattle with the earliest experiments conducted in the 1950s [26]. 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)[34].

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[53]. 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[15]. 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[19] or under seasonal heat stress conditions[20] even improving fertility over spontaneous oestrus[21] or in cows with lower (<2.75) BCS[51]. Utilization of eCG in a synchronization protocol has also a positive effect on P4 plasma levels in the ensuing oestrous cycle[51, 31].

However, to the best of our knowledge, 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. Brieûy, 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 deûned 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 PGF2á (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 -20º 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 Twine pregnancy dairy cows

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

*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

				Variables in equation					
		B	SE	Wald test	df	Sig.	Exp. (B)	95% C.I. for Exp. (B)	
optimal BUN	eCG	Ref					1	lower	upper
	Ovsynch	-0.086	0.548	0.024	1	0.876	0.918	0.314	0.314
High BUN	eCG	-0.324	0.544	0.355	1	0.551	0.723	0.249	0.249
	Ovsynch	-0.885	0.585	2.294	1	0.130	0.413	0.131	0.131
Parity	0.221	0.208	1.135	1	0.287	1.247	0.831	0.831	
Milk	-0.019	0.024	0.625	1	0.429	0.981	0.936	0.936	
Fat	-0.460	0.311	2.193	1	0.139	0.631	0.343	1.161	
Protein	0.074	0.707	0.011	1	0.917	1.077	0.269	4.307	
SCC	0.000	0.001	0.104	1	0.747	1.000	0.998	1.001	
Constant	1.094	2.617	0.175	1	0.675	2.987			

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

				Variables in equation					
		B	SE	Wald test	df	Sig.	Exp. (B)	95% C.I. for Exp. (B)	
								lower	upper
optimal BUN	eCG	Ref							
	Ovsynch	-0.122	0.548	0.050	1	0.823	0.885	0.302	2.591
High BUN	eCG	-0.646	0.557	1.344	1	0.246	0.524	0.176	1.562
	Ovsynch	-1.522	0.646	5.773	1	0.016	0.212	0.060	0.751
Parity	0.160	0.213	0.568	1	0.451	1.174	0.774	1.781	
Milk	0.004	0.026	0.026	1	0.873	1.004	0.953	1.058	
Fat	-0.462	0.325	2.016	1	0.156	0.630	0.333	1.192	
Protein	0.120	0.731	0.027	1	0.870	1.127	0.269	4.723	
SCC	0.000	0.001	0.003	1	0.956	1.000	0.998	1.002	
Constant	-0.068	2.730	0.001	1	0.980	0.934			

Table 4. Effect of eCG on P4 and E2 concentrations on days 0 and 5

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

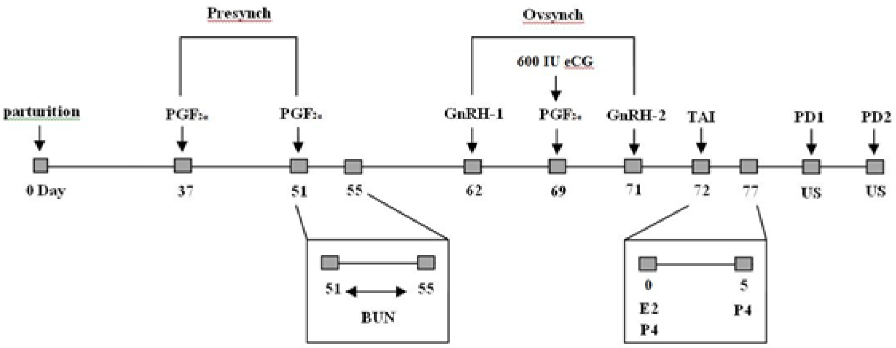

Fig. 1. Diagram of the treatments for Presynch-Ovsynch timed AI program, with or without administration of 600IU eCG on day 69. Abbreviations: BUN, blood sampling and analysis of urea nitrogen; P4, blood sampling and analysis of serum P4 concentrations; US, ultrasonography; PD, pregnancy diagnosis by 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 cow 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[33]. Urea is a product of protein breakdown and the peripheral urea nitrogen concentrations reflects protein metabolism[10]. Urea diffuses into body fluids such as blood serum and milk and equilibrates in other parts of the body including, reproductive tissues[37]. Both high and low circulating urea concentrations have been associated with reduced fertility in dairy cows, particularly increased calving to conception interval[11,33,39,57]. 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[47]. Cultured oocytes with high concentrations of urea resulted in fewer oocytes successfully progressing to metaphase II[17]. When oocytes were exposed to high levels of urea in vitro[40] or to metabolism of high protein diets in vivo[2,47], 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[47] the diameter of the preovulatory follicle or, had no effect[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[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[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[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[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[3]. High concentrations of urea in CM can cause decreased sperm motility and survival. And in addition, decreases ferny-like patterns and increases atypical, and/or stellate structures. In general, it causes reduction in CM quality[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 estus, Alkaline pH of CM is favorable for sperm motility[43], so, with increased levels of estrogen, probably CM quality improves. The eCG treatment increased dominant follicle diameter[42]. Follicles of larger diameters cause an increase in E2 concentrations[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[41], 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[1], the effects of P4 in inducing dramatic changes in the uterus, termed progestogenic changes, are essential to produce an environment compatible with embryonic growth, implantation and maintenance of pregnancy[1]. High dietary CP reduced plasma P4 concentrations in lactating cows in three of four studies[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)[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[27,35,36,54]. Higher plasma P4 levels ameliorate some of the adverse effects of BUN on fertility after AI in dairy cows[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 of pregnancy rate with fat supplementation[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[58]. Administration of eCG before ovulation, also leads to augmentation of plasma P4 levels during the oestrous cycle subsequent to the treatment[7,40,55]. 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 progestron 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

1. Arck P, Hansen PJ, Jericevic BM, Piccinni MP, Szekeres-Bartho J. Progesterone during pregnancy: endocrine-immune cross talk in mammalian species and the role of stress. *Am J Reprod Immunol*, 2007; **58**: 268-279.
2. Armstrong, D.G., McEvoy, T.G., Baxter, G., Robinson, J.J.Hogg, C.O., Woad, K.J., Webb, R., Sinclair, K.D., Effect of dietary energy and protein on bovine follicular dynamics and embryo production in vitro: associations with the ovarian insulin-like growth factor system. *Biol. Reprod.* 2001; **64**: 1624–1632.
3. Beran, J., Stadnik, L., DuchaÇcek, J., Okrouhla, M., Relationships between changes in Holstein cow's body condition, acetone and urea content in milk and cervical mucus and sperm survival. *Acta Univ. Agric. Silvic. Mendel. Brun*. 2012b; **60**: 39-48.
4. Beran j , Stadnik L., Relationships among the cervical mucus urea and acetone,accuracy of insemination timing, and sperm urvival in Holstein cows, *Animal Reproduction Science* 2013 ; **142**: 28-34.
5. Biswajit Roy, B. Brahma, S. Ghosh, P.K. Pankaj and G. Mandal, Evaluation of Milk Urea Concentration as Useful Indicator for Dairy Herd Management: A Review. Asian Journal of Animal and Veterinary Advances, 2011; **6**: 1-19.
6. Blauwiekel, R., R. L. Kincaid, and J. J. Reeves. Effect of high crude protein on pituitary and ovarian function in Holstein cows. *J. Dairy Sci.* 1986; **69**: 439-446.
7. Bodensteiner KJ, Kot K, Wiltbank MC, Ginther OJ, Synchronization of emergence of follicular waves in cattle. *Theriogenology* 1996; **45**: 1115-1128.
8. Butler, W. R., and R. D. Smith. Interrelationships between energy balance on postpartum reproductive function in dairy cattle. *J. Dairy Sci*. 1989; **72**:767-783.
9. Butler, W. R., J. J. Calaman, and S. W. Beam. Plasma and milk urea nitrogen in relation to pregnancy rate in lactating dairy cattle. *J Anim Sci* 1996; **74**: 858-865.
10. Butler, W.R., Review: effect of protein nutrition on ovrian and uterine physiology in dairy cattle. *J. Dairy Sci*. 1998; **81**: 2533–2539.
11. Butler, W.R. Nutritional Effects on Resumption of Ovarian Cyclicity and Conception Rate in Postpartum Dairy Cows; British Society of Animal Science Occasional Publication 26. Nottingham University Press: Edinburgh, UK, 2001; 133-145.
12. Canfield, R.W., Sniffen, C.J., Butler, W.R., Effects of excess degradable protein on postpartum reproduction and energy balance in dairy cattle. *J. Dairy Sci*. 1990; **73**: 2342–2349.
13. Cavalieri J, Rubio I, Kinder JE, Entwistle KW, Fitzpatrick LA. Synchronization of estrus and ovulation and associated endocrine changes in Bos indicus cows. *Theriogenology* 1997; **47**: 801–14.
14. Chenault, J. R., W. W. Thatcher, P. S. Kalra, R. M. Abrams, and C. J. Wilcox. Transitory changes in plasma progestins, estradiol, and luteinizing hormone approaching ovulation in the bovine. *J. Dairy Sci*. 1975; **58**:709–717.
15. De Rensis F and Lopez-Gatius F, Use of Equine Chorionic Gonadotropin to Control Reproduction of the Dairy Cow: A Review, *Reprod Dom Anim* 2014; **49**: 177-182.
16. DeWit, A.A., Cesar, M.L., Kruip, T.A., Effect of urea during in vitro maturation on nuclear maturation and embryo development of bovine cumulus-oocyte-complexes. *J. Dairy Sci*. 2001; **84**: 1800-1804.
17. Ferguson, J. D., D. T. Galligan, T. Blanchard, and M. Reeves. Serum urea nitrogenand conception rate: The usefulness of test information. *J Dairy Sci* 1993; **76**: 3742-3746.
18. Garcia-Bojalil, C.M., Staples, C.R., Risco, C., Savio, J.D., Thatcher,W.W., Protein degradability and calcium salts of long chain fatty acids in the diets of lactating dairy cows: reproductive responses. *J. Dairy Sci*. 1998; **81**: 1385–1395.
19. Garcia-Ispierto I, L_opez-Helguera I, Martino A, L_opez-Gatius F, Reproductive performance of anoestrous high-producing dairy cows

improved by adding equine chorionic gonadotrophin to a progesterone-based oestrous synchronizing protocol. *Reprod Domest Anim* 2012; **47**: 752– 758.

20. Garcia-Ispierto I, Rosell_o MA, De Rensis F, L_opez-Gatius F, A five-day progesterone plus eCG-based fixed-time AI protocol improves fertility over spontaneous estrus in high-producing dairy cows under heat stress. *J Reprod Dev* 2013; **59**: 544-548.
21. Garcia-Ispierto I, L_opez-Gatius F, A three day PGF2a plus eCG-based fixed— time AI protocol improves fertility over spontaneous estrus in dairy cows with silent ovulation. *J Reprod Dev* 2013; **59**: 393– 397.
22. Rabuffo, and S. K. Chandler. An in vitro model for the study of bovine endometrial physiology and pathophysiology. Page II–1 *in* Proc. 13th Int. Congr. Anim. Reprod., Sydney, Australia, 1996.
23. Goudet, G., J. Bezard, G. Duchamp, N. Gerard, and E. Palmer. Equine oocyte competence for nuclear and cytoplasmic in vitro maturation: Effect of follicle size and hormonal environment. *Biol. Reprod*. 1997; **57**: 232–245.
24. Grings, E. E., R. E. Roffler, and D. P. Deitelhoff. Response of dairy cows in early lactation to additions of cottonseed meal in alfalfa-based diets. *J. Dairy Sci*. 1991; **74**: 2580–2587.
25. Hafez, B., Hafez, E.S.E., Reproduction in Farm Animals. Maryland, USA, 2000; 495.
26. Herrick JB., Clinical observation of progesterone therapy in repeat breeding heifers. *Vet Med*, 1953; **48**: 489-490.
27. Inskeep EK, Preovulatory, postovulatory, and postmaternal recognition effects of concentrations of progesterone on embryonic survival in the cow. *Journal of Animal Science* 2004; **82**: E24-E39.
28. Ito, M., H. Iwata, M. Kitagawa, Y. Kon, T. Kuwayama, and Y. Monji., Effect of follicular fluid collected from various diameter follicles on the progression of nuclear maturation and developmental competence of pig oocytes. *Anim. Reprod. Sci*. 2008; **106**: 421-430.
29. Jordan, E. R., and L. V. Swanson. Serum progesterone and luteinizing hormone in dairy cattle fed varying levels of crude protein. *J. Anim. Sci*. 1979; **48**: 1154-1158.
30. Kung Jr., L., and J. T. Huber. Performance of high producing cows in early lactation fed protein of varying amounts, sources and degradability. *J. Dairy Sci*. 1983; **66**: 227-234.
31. Kenyon AG, Lopes G Jr, Mendonc_a LG, Lima JR, Bruno RG, Denicol AC, Chebel RC, Ovarian responses and embryo survival in recipient lactating Holstein cows treated with equine chorionic gonadotropin. *Theriogenology* 2012; **77**: 400–411.
32. Lamb, G. C., J. S. Stevenson, D. J. Kesler, H. A. Garverick, D. R. Brown, and B. E. Salfen. Inclusion of an intravaginal progesterone insert plus GnRH and prostaglandin F2±for ovulation control in postpartum suckled beef cows. *J. Anim. Sci*. 2001; **79**: 2253–2259.
33. Laven, R.A.; Drew, S.B. Dietary protein and the reproductive performance of cows. *Vet. Rec*. 1999; **145**: 687–695.
34. Mann GE, Lamming GE. The influence of progesterone during early pregnancy in cattle. *Reprod Domest Anim*, 1999; **34**: 269-274.
35. Mann GE & Lamming GE, Relationship between maternal endocrine environment, early embryo development and inhibition of the luteolytic mechanism in cows. *Reproduction* 2001; **121**: 175-180.
36. McNeill RE, Diskin MG, Sreenan JM & Morris DG, Associations between milk progesterone concentration on different days and with embryo survival during the early luteal phase in dairy cows. *Theriogenology,* 2006; **65:** 1435–1441.
37. Melendez, P.; Donovan, A.; Hernandez, J.; Barolome, J.; Risco, C.A.; Staples, C.; Thatcher, W.W. Milk, plasma, and blood urea nitrogen concentrations, dietary protein, and fertility in dairy cattle. *J. Am. Vet. Med. Assoc*. 2003; **223**: 628–634.
38. Mermillod, P., B. Oussaid, and Y. Cognie. Aspects of follicular and oocyte maturation that affect the developmental potential of embryos. *J. Reprod. Fertil. Suppl*. 1999; **54**: 449-460.
39. Moore, D.A.; Varga, G. BUN and MUN: Urea nitrogen testing in dairy cattle. Compendium 1996; **18**: 712-720.
40. Murphy BD, Martinuk SD, Equine chorionic gonadotrophin. *Endocr Rev* 1991; **12**: 27–44.
41. Ocon, O.M., Hansen, P.J., Disruption of bovine oocytes and preimplantation embryos by urea and acidic pH. *J. Dairy Sci*. 2003; **86**: 1194–1200.
42. Pacala N, Corin N, Bencsik I, Dronca D, Cean A, Boleman A, Caraba V, Papp S, Stimulation of the reproductive function at cyclic cows by ovsynch and PRID/eCG. *Anim Sci Biotech* 2010; **43**: 317–320.
43. Pattabiraman, S.R., Venkataswamy, V., Thangraj, T.M., Physicochem- ical properties of oestrual mucus of cows. *Indian Vet. J*. 1967; **10**: 413–417.
44. Perry GA, Smith MF, Lucy MC, Green JA, Parks TE, MacNeil MD, *et al*. Relationship between follicle size at insemination and

pregnancy success. *Proc Natl Acad Sci USA* 2005; **102**: 5268–73.

45. Rajala-Schultz, P.J., Saville,W.J.A., Frazer, G.S.,Wittum, T.E., Association between milk urea nitrogen and fertility in Ohio dairy cows. *J. Dairy Sci.* 2001; **84**: 482-489.
46. Roffler, R. E., and D. L. Thacker. Influence of reducing dietary crude protein from 17 to 13.5% on early lactation. *J. Dairy Sci.* 1983; **66**: 51-58.
47. Sà Filho MF, Penteado L, Reis EL, Gimenes LU, Baruselli PS. Cyclicity and eCG treatment effects on follicular dynamics and conception rate in Nellore heifers treated with norgestomet auricular implant and estradiol benzoate [Abstract]. *Acta Sci Vet* 2005; **33**(Suppl 1): 265.
48. Sinclair, K.D., Kuran, M., Gebbie, F.E.,Webb, R., McEvoy, T.G., Nitrogen metabolism and fertility in cattle: II. Development of oocytes recovered from heifers offered diets differing in their rate of nitrogen release in the rumen. *J. Anim. Sci*. 2000; **78**: 2670–2680.
49. Small JA, Colazo MG, Kastelic JP, Mapletoft RJ. Effects of progesterone presynchronization and eCG on pregnancy rates to GnRHbased, timed-AI in beef cattle. *Theriogenology* 2009; **71**: 698–706.
50. Sonderman, J. P., and L. L. Larson. Effect of dietary protein and exogenous gonadotropin-releasing hormone on circulating progesterone concentrations and performance of Holstein cows. *J. Dairy Sci.* 1989; **72**: 2179–2183.
51. Souza AH, Viechneski S, Lima FA, Silva FF, Ara_ujo R, B_o GA, Wiltbank MC, Baruselli PS, Effects of equine chorionic gonadotropin and type of ovulatory stimulus in timed-AI protocol on reproductive responses in dairy cow. *Theriogenology* 2009; **72**: 10–21.
52. Staples, C. R., C. M. Garcia-Bojalil, B. S. Oldick, W. W. Thatcher, and C. A. Risco., Protein intake and reproductive performance of dairy cows: a review, a suggested mechanism, and blood and milk urea measurements. Pages 37–51 in Proc. 4th Annu. Florida Ruminant Nutr. Symp., Univ. Florida,n Gainesville, 1993.
53. Stewart F, Allen WR, Biological functions and receptor binding activities of equine chorionic gonadotrophins. *J Reprod Fertil* 1981; **62**: 527–536.
54. Stronge AJ, Sreenan JM, Diskin MG, Mee JF, Kenny DA & Morris DG, Post-insemination milk progesterone concentration and embryo survival in dairy cows. *Theriogenology* 2005; **64**: 1212–1224.
55. Takahashi T, Hirako M, Patel OV, Shimizu M, Hasegavwa Y, Plasma steroid profiles following follicle-stimulating hormone or equine chorionic gonadotrophin injection in cows chronically treated with gonadotrophin releasing hormone agonist. *J Vet Med Sci* 2002; **64**: 731–734.
56. Vasconcelos, J. L. M., R. Sartori, H. N. Oliveira, J. G. Guenther, and M. C. Wiltbank. Reduction in size of the ovulatory follicle reduces subsequent luteal size and pregnancy rate. *Theriogenology* 2001; **56**:307–314.
57. Wathes, D.C.; Bourne, N.; Cheng, Z.; Mann, G.E.; Taylor, V.J.; Coffey, M.P. Multiple correlation analyses of metabolic and endocrine profiles with fertility in primiparous and multiparous cows. *J. Dairy Sci.* 2007; **90**: 1310–1325.
58. Wiltbank MC, A.H. Souza, J.O. Giordano, A.B. Nascimento *et al,* Positive and negative effects of progesterone during timed AI protocols in lactating dairy cattle. *Anim Reprod,* 2012; **9**(3): 231-241.