The interaction of blood urea nitrogen and semen type in a fixed time AI program in Angus cross beef cows–a pilot study

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Abstract

Cows fed excess dietary protein had increased blood urea (BUN), altered uterine fluid composition, decreased uterine pH, and reduced fertility. The objective of the study was to examine the effect of BUN concentrations at the day of breeding on the fixed time artificial insemination (AI) pregnancy rate in Angus cross beef cows inseminated with fresh extended or frozen thawed semen. Angus cross beef cows (N=266) from seven locations were synchronized with a CO-Synch + CIDR protocol for fixed time AI. Cows were inseminated with either fresh extended (3 million sperm, Caprogen® extender) or frozen thawed semen (20 million sperm; egg yolk extender) from two AI sires. On the day of AI, blood samples were collected for BUN analysis. In the study population, there were 165 cows with ≤ 18 mg/dL (mean 11.6) and 101 cows with > 18 mg/dL (mean 23.2) BUN concentrations. Accounting for significant variables such as AI sire ($P<0.001$) and semen type by BUN level interaction ($P<0.05$) in the model, there were no differences in the fixed time AI pregnancy between BUN groups ($P>0.05$). However, the fixed time AI pregnancy rate was different between fresh and frozen semen, 61.1% and 52.4%, respectively ($P<0.05$). The AI pregnancy rates were significantly different between sires 1 and 2, 66.7% and 44.0%, respectively ($P<0.05$). A semen type by BUN concentration interaction for AI pregnancy was recorded. No AI sire by BUN concentration or AI sire by semen type interactions were recorded. There was a quadratic relationship observed for locations' mean BUN concentrations with locations' mean AI pregnancy rates. Lower and higher mean location BUN concentrations had reduced AI pregnancy. Cows with >18% mg/dL BUN concentrations inseminated with fresh semen had a 61.2% AI pregnancy rate. Our findings suggest that the negative effect of increased BUN concentrations may be overcome by utilizing fresh semen extended in an extender with catalase (Caprogen®) in a fixed time AI programs.

Introduction

Previous research has shown that cows fed excess dietary protein had increased blood urea, altered uterine fluid composition, decreased uterine pH, and reduced fertility.[1-4] Dairy cows with BUN and milk urea nitrogen (MUN) >19 mg/dL showed a 20 percentage point decrease in AI pregnancy rate.[2] Rhoads et al showed that short-term increase in plasma urea nitrogen (PUN) can exert direct effects on the uterine environment by decreasing uterine pH.[4] Nitrogenous waste products (e.g., NH_3 and urea) are toxic to bovine gametes and/or embryos and have been suggested to play a role in reproductive inefficiency.[1] High PUN concentrations changed the follicular, oviductal and uterine environment, and decreased embryo viability through effects exerted on the oocyte or embryo.[3] Also the PUN status of the donor cows affected pregnancy rate following embryo transfer.[3] Cows grazing spring pastures in Virginia are exposed to very high protein diets which may have the potential to decrease pregnancy rates (http://pubs.ext.vt.edu/news/livestock/2010/06-07/LU_06-01-10-3.html). The objective of the study was to examine the effect of BUN concentrations at the day of breeding on the fixed time AI pregnancy rate in Angus cross beef cows inseminated with fresh and frozen semen.

Materials and methods

Angus cross beef cows (N=266) from seven locations were synchronized with a CO-Synch + CIDR protocol (Figure 1) for fixed time AI (FTAI). Briefly, cows enrolled in the study received 100 μg of gonadorelin diacetate tetrahydrate im (GnRH; Cystorelin® sterile solution, Merial, Athens, GA) and 1.3 g progesterone in an intra-vaginal insert (CIDR; Eazi-Breed™ CIDR cattle insert®, Pfizer Animal Health, New York, NY) on Day 0. Additionally, body condition scores (BCS) of cows in the study were recorded

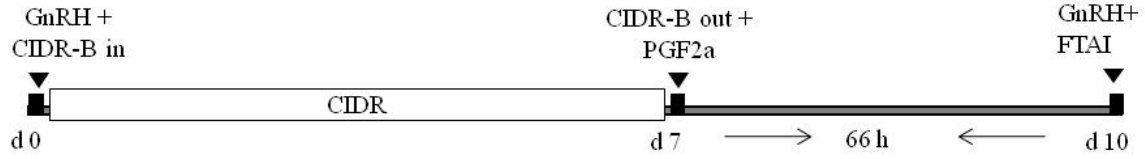
on Day 0. On Day 7, the CIDR was removed, and 25 mg of dinoprost ($PGF_2\alpha$; Lutalyse® sterile solution, Pfizer Animal Health, New York, NY) was administered im. Cows were inseminated with either fresh extended or frozen thawed semen, at a fixed-time on Day 10, 66 h after CIDR removal and received 100 μg of GnRH at this time.

Two mature, known highly fertile Angus bulls from a commercial stud center were used in this study. Prior to initiation of the study, semen samples of each sire were screened for presence of *Mycoplasma sp*. weekly for three consecutive weeks according industry guidelines (Certified Semen Services, Columbia, MO). On collection day two ejaculates were collected serially by artificial vagina and pooled within bull. The pooled semen from each bull was assessed for motility and concentration and then divided into two aliquots.

Aliquot one was extended using Caprogen® diluent (LIC, Hamilton, New Zealand) to a concentration of 3×10^6/ 0.25 mL French straw. Fresh semen samples were packaged in 0.25 mL French straws that are specially designed for use with non-frozen semen (IMV Technologies, L'Aigle, France). Straws were stored in polystyrene foam boxes at ambient temperature (approximately 20 to 25°C) until insemination. Aliquot two was extended using an egg-yolk-glycerol extender to a concentration of 20×10^6/ 0.5 mL French straw and frozen in liquid nitrogen vapor (-196°C). Semen straws were shipped from the stud center to the breeding locations and cows were artificially inseminated within 48 hours of collection. To meet the stud center standard, fresh semen samples were evaluated each morning to confirm sufficient maintenance of motility (≥ 60 %) and acrosomal integrity (≥ 70 %) prior to use in AI. Frozen semen was assessed for post-thaw motility after 0 and 3 h of incubation at 37°C (> 60 % and > 25 %, respectively) and for the percentage of intact acrosomal membranes after 3 h of incubation at 37°C (> 60%).

Cows were randomly assigned to fresh semen and frozen semen groups and two AI sires. Blood samples were collected by coccygeal venipuncture for BUN analysis on the day of FTAI. Pregnancy diagnosis was performed 60 days after insemination using transrectal ultrasonography (Sonosite 180 plus, Sonosite Inc., Bothell, WA). Artificial insemination pregnancy rate was calculated by number of cows diagnosed pregnant divided by number of cows inseminated.

Figure 1.



Statistics

Mixed model (GLIMMIX procedure of SAS version 9.12, SAS Institute Inc., Cary, NC) was used to determine the effect BUN concentration categories (≤ 18 mg/dL vs. >18 mg/dL) on FTAI. Other variables included in the model were semen type (fresh, 3×10^6 vs. frozen, 20×10^6), AI sires (1 vs. 2), BCS (<5, 5 to 6, >6; 1 = emaciated; 9 = obese), days postpartum at initiation of synchronization (30 to 60, 61 to 80, >80), age of dam (2, 3 to 6 and > 6 years). Locations were treated as random effect. A *P* value of ≤ 0.05 was considered significant. The model was created by manual backward stepwise elimination procedure (P > 0.10 for exclusion). Only variables of interest (BUN categories) and statistically significant variables were retained in the final model.

Results

Among the study population, 132 cows were inseminated with semen from Sire 1 and 134 cows were inseminated with semen from Sire 2. There were 130 and 136 cows inseminated with fresh and frozen semen, respectively. In the study population, there were 165 cows with ≤ 18 mg/dL (mean 11.6) and 101 cows with > 18 mg/dL (mean 23.2) BUN concentrations. Accounting for significant variables

such as AI sire ($P<0.001$), and semen type × BUN level interaction ($P<0.05$) in the model, there were no differences in the FTAI pregnancy rate between BUN groups ($P>0.05$). However, the FTAI pregnancy rate was different between semen types ($P<0.05$; Table 1). The AI pregnancy rates were significantly different between sire 1 and 2, 64.7% and 45.4%, respectively ($P<0.05$). A semen type × BUN concentration interaction for AI pregnancy was recorded (Figure 2). There were no AI sire × BUN concentration or AI sire × semen type interactions observed. The BUN concentration affected the AI pregnancy rate for frozen semen (Figure 2). There was a quadratic relationship observed for mean location BUN concentrations and location AI pregnancy rates (Figure 3). Lower and higher mean location BUN concentrations had reduced AI pregnancy rates.

Discussion

In the present study, the pregnancy rate following insemination with fresh semen and frozen semen was not different despite differences in average sperm concentration in fresh versus frozen straws. The similarity in pregnancy rates may be attributable to both survivability of fresh sperm and the impaired functional and structural parameters of sperm after the freeze-thaw process.[5] It is possible that frozen semen is less likely either to survive long enough in the female reproductive tract or to maintain competency in fertilization, as compared to fresh extended semen.[6] A plateau theory for the impact of increasing sperm numbers for most AI sires on fertility has been proposed.[7] Beyond this plateau number no increase in fertility results. Our results suggest that this plateau had been reached with both fresh and frozen semen.

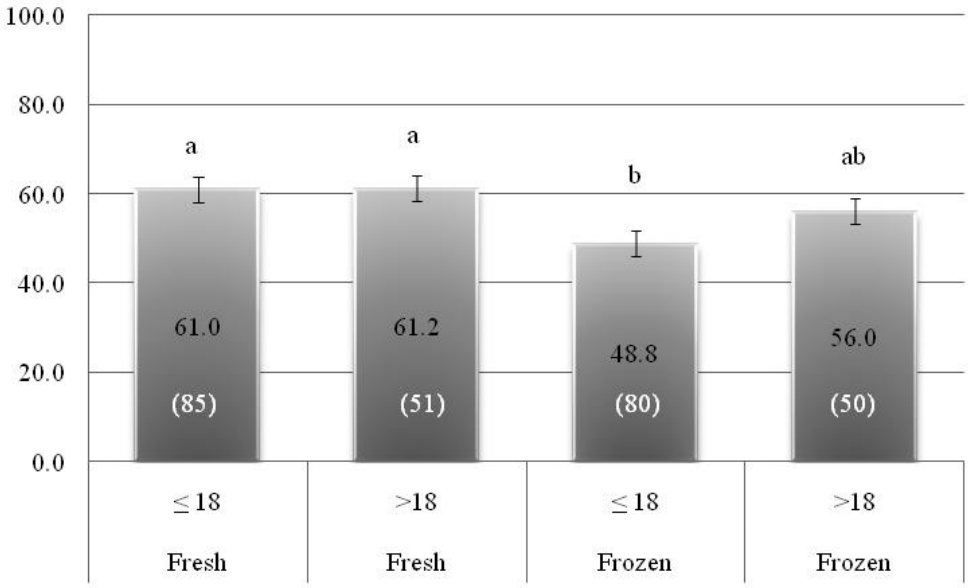
The results from this study indicated that cows with altered BUN concentrations inseminated with frozen semen had numerically reduced AI pregnancy rates. Sperm binding to oviductal epithelium preserves their fertility during storage in the sperm reservoir.[8] Cows with an increased number of sperm in the sperm reservoir resulted in increased risk of becoming pregnant.[9] It has been demonstrated that sperm viability is maintained by preventing premature capacitation and the suggested mechanism is presence of catalase in the bovine oviduct.[10,11] Caprogen® diluent is enriched with catalase. Therefore it is reasonable to expect that the fresh semen extended with Caprogen® diluent provided a better environment which facilitated a higher number of sperm in the sperm reservoir.[12] It has been demonstrated that nitrogenous waste products (e.g., NH3 and urea) are toxic to bovine gametes and/or embryos and have been suggested to play a role in reproductive inefficiency. Cows in this study with > 18 mg/dL BUN concentrations inseminated with fresh semen had a 61.2% AI pregnancy rate. So it is notable that the negative effect of increased BUN concentrations may be overcome by utilizing fresh semen extended in an extender with catalase (Caprogen®) in FTAI programs.

Table 1. The effect of semen type and BUN level on FTAI pregnancy rate in beef cows synchronized with CO-Synch+CIDR protocol[*]

Parameter	N	Treatment Groups	Mean AI-PR[†]
BUN levels	165	≤ 18 mg/dL (Mean 11.6)	55.0[a]
	101	> 18 mg/dL (Mean 23.2)	58.6[a]
Semen type	136	Fresh semen (3×10^6/straw)	61.1[a]
	130	Frozen semen (20×10^6/straw)	52.4[b]
Sires	132	1	45.4[a]
	134	2	64.7[b]

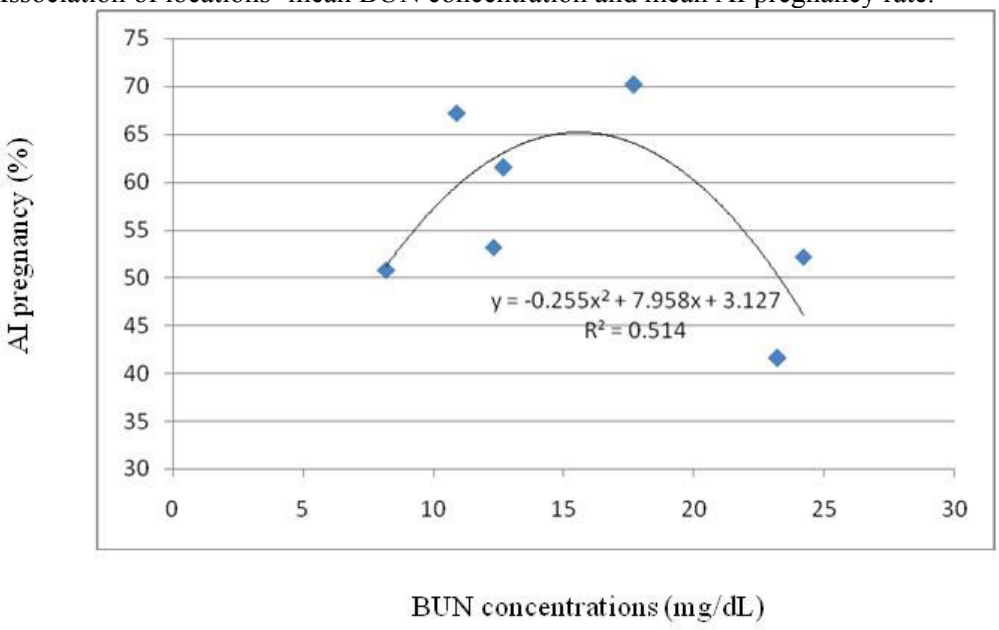
[*]Refer to Figure 1 for protocol;
[†]Accounted semen type by BUN level interaction (P<0.05)
[a,b]Numbers with no common superscript were different (P < 0.05)
AI-PR – pregnancy rate for fixed time AI.
CIDR – Controlled Internal Drug Release (intravaginal progesterone insert).
BUN – Blood urea nitrogen.

Figure 2. Fixed time AI pregnancy rate among semen type and BUN concentration (mg/dL) groups in beef cows



[ab]Bars with no common superscript were different (P < 0.05)

Figure 3: Association of locations' mean BUN concentration and mean AI pregnancy rate.



References

1. Butler WR: Review: effect of protein nutrition on ovarian and uterine physiology in dairy cattle. J Dairy Sci 1988;81:2533-2539.
2. Butler WR, Calaman JJ, Beam SW: Plasma and milk urea nitrogen in relation to pregnancy rate in lactating dairy cattle. J Anim Sci 1996;74:858-865.
3. Rhoads ML, Rhoads RP, Gilbert RO, et al: Detrimental effects of high plasma urea nitrogen levels on viability of embryos from lactating dairy cows. Anim Reprod Sci 2006;91:1-10.
4. Rhoads ML, Gilbert RO, Lucy MC, et al: Effects of urea infusion on the uterine luminal environment of dairy cows. J Dairy Sci 2004;87:2896-2901.
5. Parks JE, Graham JK: Effects of cryopreservation procedures on sperm membranes. Theriogenology 1992;38:209-222.
6. Vishwanath R, Pitt CJ, Shannon P: Sperm numbers, semen age and fertility in fresh and frozen bovine semen. Proc NZ Soc Anim Prod 1996;56:31-34.
7. Saacke RG: Sperm morphology: its relevance to compensable and uncompensable traits in semen. Theriogenology 2008;70:473-478.
8. Lefebvre R, Chenoweth PJ, Drost M, et al: Characterization of the oviductal sperm reservoir in cattle. Biol Reprod 1995;53:1066-1074.
9. Hunter RHF: Sperm transport and reservoir in the pig oviduct in relation to time of ovulation. J Reprod Fertil 1981;63:109-117.
10. Lapointe S, Sullivan R, Sirard M-A: Binding of a bovine oviductal fluid catalase to mammalian spermatozoa. Biol Reprod 1998;58:747-753.
11. Lapointe, S., Sirard M-A: Catalase and oviductal fluid reverse the decreased motility of bovine sperm in culture medium containing specific amino acids. J Androl 1998;19:31-36.
12. Vishwanath R, Shannon P: Do sperm cells age? A review of the physiological changes in sperm during storage at ambient temperature. Reprod Fertil Dev 1997;9:321-331.