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Relation between some hematological parameters and reproductive efficiency in Holstein – heifers in Egypt

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ABSTRACT

This study was undertaken to examine some blood parameters and minerals in serum in relation to estrus and early pregnancy (on 28th day) in nulliparous heifers. Blood samples were obtained from 42 nulliparous heifers fed on TMR (Total Mixed Ration) on the day of AI and 28 days after AI. Sera were analyzed for blood urea nitrogen (BUN), glucose, zinc (Zn), copper (Cu), and selenium (Se) concentrations by Atomic absorbent spectroscopy. First service per conception and pregnancy rates were 64.3% (27/42) and 66.6% (28/42), respectively. The pregnancy was confirmed and diagnosed on the 28th day by using ultrasound. There was noticeable increase in blood glucose, Zn, and Se levels in pregnant heifers (28/42) on both day of AI and 28th day after AI and higher than their levels in non-pregnant heifers (14/42). While there was clear increase in BUN level in none conceived heifers on the day of AI (estrus) and higher than its level in the conceived heifers. There was a decrease in Cu level in conceived heifers on both the day of AI and the 28th day after AI lower than its level in non-conceived heifers. It is concluded that blood glucose, Zn, and Se are important for fertility and conception in nulliparous heifers, while increase of BUN and Cu during estrus are limiting the fertility and conception in heifers.

1. INTRODUCTION

There is a relationship between some minerals and other hematochemical parameters and conception in heifers and cow (Small.e.al, 1996). Diagnoses of mineral deficiencies or toxicities are difficult and generally require knowledge of the nutrient composition of the diet, as well as at least one measure from body tissue. Concentrations of minerals in blood serum or plasma are generally related to intake, but are influenced by sex, breed, age, and reproductive status e.g. pregnancy or lactation (Underwood 1981). Minerals are loosely classified as macro or micro minerals depending on the relative amounts needed or present in the body. Macro minerals include calcium, phosphorus, magnesium, potassium, sulphur, sodium and chloride. Cobalt, copper, iodine, iron, manganese, molybdenum, selenium and zinc are considered micro or trace minerals (Ferguson et al., 1996). Rations that contain a high percentage of forage usually supply adequate amounts of calcium but may be low in phosphorus. However, rations high in grain contain adequate phosphorus but may be deficient in calcium and other minerals (Yasothai R., 2014). Micro or trace mineral deficiencies are associated with soil type and are usually geographically related. Abnormal levels of some minerals such as iron and cobalt do not usually cause a problem with reproduction. Other minerals, including those that follow, can significantly affect reproduction (Yaremcio, 2000). Blood urea nitrogen is synthesized in the liver and can have variable concentrations without causing any adverse effects to the fertility of cattle. However, High dietary protein (nitrogen) intake resulting in BUN of greater than 19 to 20 mg/dL has been associated with an altered uterine environment and decreased fertility (reduced conception rate, decreased pregnancy rate) (Elrod and Butler,1993; Elrod et al., 1993; Ferguson et al., 1993; Butler et al., 1996). Elevated BUN concentration decreases uterine pH, which is thought to have a negative effect on embryo development and implantation (Elrod and Butler, 1993). Blood glucose is the main indicator of energy. Carbohydrates in the form of glucose are the principal source of energy for the life processes of the mammalian cell. All cells require a constant supply of this indispensable nutrient, and only relatively small changes may be tolerated without adverse effects upon the health of the animal (Sulieman et al., 2017).

Energy is the first limiting dietary factor for cows in early lactation and heifers' fertility. Energy demands in the form of milk output and body maintenance exceeds energy inputs in early postpartum and also somewhat significantly exceed in ovulation, conception and early pregnancy in heifers. Pandey.e.al, 2015 concluded that, exogenous supplementation of different hormones like GnRH, hCG and progesterone influenced the levels of glucose and cholesterol in blood, which possibly helped in the biosynthesis of progesterone by the luteal cells during early phases of embryonic development and improved conception rate in

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crossbred cattle. In which there were significantly higher plasma glucose and cholesterol level in pregnant animals as compared to non-pregnant animals. Plasma glucose level had significantly increased between day 0 and day 5 of estrous cycle compared to control group of pregnant animals.

Green e.al. (2012) illustrated that Fetal growth within cows conceiving at first insemination compared to second or third insemination was more rapid and was associated with greater blood glucose and IGF1 early *postpartum* (before day 30). Blood glucose (mg/dl) levels decreased significantly as pregnancy advanced in both heifers and cows. Decrease in blood glucose as gestation advances may be due to utilization of glucose for the development and growth of the fetus (Padodar.e.al, 2014). Zinc is known to be essential for proper sexual maturity (development of secondary sexual characteristics), reproductive capacity (development of gonadal cells) in males and all reproductive events (estrus, pregnancy and lactation), more specifically with onset of estrus in female. Among these decreased fertility and abnormal reproductive events are of prime importance in females (Sathish Kumar, 2003) whereas in male poor semen quality, reduced testicular size and libido are the usual clinical findings (Mass, 1987). Apart from this zinc has a critical role in repair and maintenance of uterine lining following parturition and early return to normal reproductive function and estrus (Greene et al., 1998).

Favier e.al. (2002), Beletskaya el al (2014) and Neve (1998) showed that fetal growth restriction and embryo death in dairy cows are directly connected with zinc deficiency. The safety margin (difference between normal requirement and toxic dose) for selenium is so narrow that its deficiency is quite rare in farm animals than its toxicity, but causes weak, silent or irregular estrus, retained fetal membranes, early embryonic death, still birth or weak offspring and abortions in females (Randhawa and Randhawa, 1994) and reduced sperm mortality in males.

The selenium supplementation at breeding season has a direct positive effect on conception rate from the first service (McClure et al., 1986).

Copper is one of the important mineral for reproduction point of view as such its deficiency is reported to be responsible for early embryonic death and resorption of the embryo (Miller et al., 1988), increased chances of retained placenta and necrosis of placenta (O'Dell, 1990) and low fertility associated with delayed or depressed estrus (Howell and Hall, 1970).

2. MATERIAL AND METHODS

2.1. Animals

Forty-two nulliparous heifers aging from 393-669 days and weighing about mean 369 kg B. Wt. in a private farm (heifers, station) in Gamasa, Al-Ismailia province. All animals had well nutrition and water supply well sheltered. All animals are vaccinated according to the vaccination program of the station. All animals are reared in the station coming from the dairy cow's station after parturition and weaning, and moreover also had registered records per each head. All animals are fed on TMR (Total Mixed Ration) with balanced composition**.** Heifers are classified into 2 groups acc to conception rate into: *Group A*, include 28 conceived heifers and *Group B*, include 14 failed or non-conceived heifers.

2.2. Blood sampling

At the time of insemination and 28 d later, blood samples were collected from all animals by jugular vein puncture into nonheparinized (20-mL) evacuated tubes. Blood samples were refrigerated overnight to allow clot formation, then centrifuged 3000 rpm. for 10 min and sera removed and stored frozen till the further examination. The serum was examined for BUN, Blood Glucose, Zn, Cu, Se concentration (by atomic absorbent spectroscopy).

2.3. Ultrasound

Ultrasound diagnosis and confirmation pregnancy was done by using ultrasound (Sonoscape with 8 Mega Hz trans rectal probe) on the 28th day after AI.

2.4. Statistical analysis

The obtained data are tabulated and statistically analyzed, where appropriate, by using the SPSS program (1997) according to El-Naggar (1997).

3. RESULTS

As presented in table 1, a noticeable difference has been found regarding the BUN (14.26 ±1.45; 19.63±1.07), glucose (54.11±5.94; 41.91± 2.84), Cu (10.76 ± 0.60; 12.75 ± 0.53) and Se (5.32± 0.56; 4.71± 0.44) between the conceived and non-conceived heifers respectively at the day of AI. While, no difference was found between the 2 groups regarding the Zn level (11.56± 0.49; 11.45±0.32).

As presented in table 2, a noticeable difference has been found regarding the BUN (33.75 ± 5.57; 19.05±0.99), glucose (61.65 ± 4.54; 41.13±2.67), Cu (9.42± 0.78; 12.84±0.44) and Se (6.29± 0.70; 4.66 ±0.65) between the conceived and non-conceived heifers respectively at the day 28 after AI. While, no difference was found between the 2 groups regarding the Zn level (12.53± 0.43; 11.39 ±0.3)

Table 1 Serum levels of blood parameters at the day of AI in the conceived and non-conceived nulliparous heifers (Mean ± SD).

	BUN (mg /dl)	Glucose (mg/dl)	Zn (μmol/l)	Cu (μmol/l)	Se (μmol/l)
Group A	14.26 ±1.45	54.11± 5.94	11.56± 0.49	10.76 ± 0.60	5.32± 0.56
Group B	19.63± 1.07	41.91± 2.84	11.45± 0.32	12.75 ± 0.53	4.71± 0.44

Table 1 Serum levels of blood parameters at the 28 days after AI in the conceived and non-conceived nulliparous heifers (Mean ± SD).

	BUN (mg /dl)	Glucose (mg/dl)	Zn (μmol/l)	Cu (μmol/l)	Se (μmol/l)
Group A	33.75 ± 5.57	61.65 ± 4.54	12.53± 0.43	9.42± 0.78	6.29± 0.70
Group B	19.05± 0.99	41.13± 2.67	11.39 ±0.3	12.84± 0.44	4.66 ±0.65

4. DISCUSSION

In spite of that BUN is synthesized by liver, it has a relation and indirect effect on the reproductive efficiency and fertility in cattle (Elrod and Butler, 1993; Elrod et al., 1993; Ferguson et al., 1993; Butler et al., 1996). In this study, the measurement of BUN in the serum obtained from the blood collected from the jugular vein of the nulliparous heifer at the estrus or particularly the day of service by AI, was revealed that the overall Mean ± SD of all heifers in both groups (n=42) was 15.09 ± 4.56 mg/dl and this result came in appointment with the results of Tshuma et al., 2010 in Bonsmara heifers (n=396) (Overall BUN = 5.27 ± 1.80 mmol/l ≈ 14.98 ± 5.04 mg/dl). But on the classification of heifers in 2 groups according to the conception or pregnancy, it was showed that the level of BUN had been noticeably increased in non-conceived heifers group (14/42) in the day of AI with mean concentration 19.63±1.072 mg/dl higher than that in the conceived heifers (28/42) (Mean±SD was14.26±1.45 mg/dl) , thus also come in a harmony with the results of Elrod and Butler,1993; Elrod et al., 1993; Ferguson et al., 1993; Butler et al., 1996; and Tshuma et al., 2010 in their different studies on cattle was showing that the BUN increase in the non-fertile females over the fertile ones. However, Elrod and Butler (1993), Elrod et al. (1993), Ferguson et al. (1993), Butler et al. (1996), stated that high dietary protein (nitrogen) intake resulting in BUN of greater than 17 to 18 mg/dl has been associated with an altered uterine environment and decreased fertility (reduced conception and pregnancy rates). Although that all heifers in this study was fed on the same TMR (total mixed ration), some individuals have high BUN concentration in their blood and this explained due to individual variation and genetic differences in the nitrogen utilization efficiency, metabolism of ammonia and kidney excretion of BUN according to Schoeman (1989), Stoop et al. (2007), Bouwman et al. (2010), and Hossein-Zadeh and Ardalan (2011). On the other side, there was a very significant increase in BUN concentration in early pregnancy (28 days) with mean 33.75±5.57 mg/dl over than its mean concentration in the day of AI (14.26±1.45 mg/dl) in the same group of conceived heifers, and these results agreed with Abdullah et al. (2017), who stated that: The plasma BUN concentration remained at a significantly higher level during day 0 to day 20 post-AI in pregnant crossbred cows than in the non-pregnant group, and maximum concentration was observed on day 28 post-AI (38.34 ± 2.70 mg/dl). This may indicate that the source of BUN is fetal kidney.

Opposite to our results, Ali et al. (2014), reported that BUN level was considerably lower in non–pregnant group as compared to pregnant group on day 1 (34.19±1.41 mg/dl vs. 40.34±2.00 mg/dl). Mean values of blood urea concentration for non–pregnant and pregnant groups on day 21 were 36.18±1.42 mg/dl and 42.22±2.19 mg/dl, respectively.

The blood glucose was shown also to be important blood parameter in the reproductive efficiency of nulliparous heifers. In this study, the mean blood glucose level was obviously increased in both day of AI and 28 days later in the group of conceived heifers (54.1±5.94 and 61.65±4.55 mg/dl, respectively) as compared with the group of non-conceived heifer in day of AI and after 28 days (41.91±2.84 and 41.13±2.67 mg/dl, respectively) and this result come in touch with Pandey et. Al. (2015) and Plym Forshell et. Al. (1991). But within the subdivided groups, there was noticeable increase in blood glucose level at the 28th day of pregnancy over than its level in the day of AI in the conceived heifers (61.65±4.55 and 54.1±5.94 mg/dl, respectively). This is due to great demand for more energy for beginning the fetal development in the early pregnancy and afterward it decease significantly with pregnancy advance due to higher consumption. This agreed with Pandey et. Al. (2015), Padodar et. Al. (2014), Green et. al. (2012), and Plym Forshell et. al. (1991). Our results come in agreement with Ali et al. (2014), who said that glucose level decreased in early pregnancy at 28th day of pregnancy when compared to non-pregnant animals in which blood glucose concentration exhibited relatively lower in contrast to non–pregnant cows on day–1 (44.22±0.79 mg/dl and 40.88±1.07 mg/dl, respectively). Similarly, the blood glucose content measured on day 21 (post–insemination) was higher in non–pregnant group (44.86±0.97 mg/dl) as compared to pregnant group (40.15±1.24 mg/dl).

The present study reveals a positive relation between the blood glucose level and conception and pregnancy in heifers achieving the harmony with Pandey et. al. (2015), Padodar et. al (2014), Green et. al. (2012), and Plym Forshell et. al. (1991) while disagree with De Silva et. al. (1981) and Ali et. al. (2014).

There were obvious increase in zinc and selenium on both day of AI and day 28 later in the group of conceived heifers (11.56±0.49 and 12.53±0.43 µmol/l, respectively) as compared with the group of failed heifers (11.45±0.32 and 11.39±0.30 µmol/l, respectively). This result is coordinated with that reported with Small et. al. (1996) and Shabunin et. al. (2017) in their zinc level. In addition to McClure et. al. (1986), who proved an improvement in conception rate at first service following selenium supplementation. These results prove that both zinc and selenium are very important trace elements for estrus, ovulation, conception, and fetal development in cattle. This is agreeing with Kumar (2003), Favier et. al. (2002), Beletskaya et. al. (2014), and Neve (1998) in their zinc results.

In group A of conceived heifers, there was another relation has been found between early pregnancy and both Zn and Se serum concentration. In which the serum levels of both Zn and Se were markedly high on the 28th day of pregnancy as compared to the day of AI (11.56±0.49 and 12.53±0.43 µmol/l, respectively). This positive relationship indicate that Zn and Se are so important for the fetal development, and this what was proved by Chen et. al. (2015), who found that there was developmental delay and embryo death in dairy cows at early gestation stages as consequences of zinc deficiency and disorders of its metabolism. Also, it matched to Shabunin et. al. (2017), who showed that zinc blood level in cows of fetal growth restriction group on the 28th–32nd and 38th–45th days of gestation was lower by 17.2% and 10.9%, while for those with embryo death it was lower by 25.5% and 23.1%, respectively, in comparison with control group. Shabunin et. al. (2017), also showed that selenium blood level in cows of fetal growth restriction decreased by 26.2% on the 28th–32nd days of gestation and in animals of fetal death by 29.1% in comparison with the control group (pregnant with normal fetal development).

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