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# Effect of calcium and magnesium administration on serum and follicular fluid mineral and protein profiles in buffaloes

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Apart from X and Y chromosome bearing spermatozoa, sex ratios can also be influenced by maternal diet and body condition (Green *et al*. 2008). A significant variation from the expected 1:1 birth sex ratio in many mammalian species were observed based on altering pre-conceptual maternal environment (Trivers and Willard 1973, Stolkowski and Lorrain 1980, Rosenfeld and Roberts 2004, Grant and Chamely 2010). In rats and mice, feeding of high sodium and potassium cationic elements for 2–3 weeks prior to ovulation resulted in more male pups (Chandraju *et al*. 2011, Clint *et al*. 2013), whereas, feeding of diet having high ratio of calcium and magnesium resulted in more female pups (Vahidi and Sheikhha 2007, Celik *et al*. 2003). The external administration of certain minerals, and high energy and protein diets prior to ovulation and around conception are proposed to change the follicular fluid protein profiles and altered sex ratio at birth. To address these mechanisms, a pilot study was initiated by administration of calcium magnesium borogluconate via subcutaneous route to assess the role of these minerals on the changes in buffalo serum and follicular fluid minerals and protein patterns.

*Experimental animals and serum sample collection:* The study was carried out at the experimental livestock unit of the Institute. Female adult buffaloes (7) were separated into 2 groups, treatment group (5) was administered with calcium (1.86%) magnesium (5%) borogluconate (20 ml subcutaneous route) during one complete estrous cycle, whereas control group (2) received placebo. Blood samples were collected by jugular venipuncture daily and the serum fraction was separated by centrifugation at 6,000 g for 25 min and re-centrifugation at 6,500 g for 10 min to remove further traces of blood cells. Aliquots of serum for mineral estimation were stored at –20ºC till further analysis.

*Follicular fluid collection:* Ultrasound guided trans-vaginal fine needle aspiration method was adopted for follicular fluid collection in buffaloes. At estrus, coinciding the end of calcium and magnesium administration for a period of one cycle, the ovulatory dominant follicles were aspirated. The follicular fluid samples were centrifuged at 6,000 g for 25 min and again at 6,500 g for 10 min to remove cell debris, if any. Aliquots of clear follicular fluid for mineral estimation and protein profile were stored at –20ºC till further analysis.

*Minerals estimation:* The level of minerals (magnesium, copper, boron) was estimated in serum and follicular fluid using spectrophotometry.

*2-D gel electrophoresis of follicular fluid proteins:* The follicular fluid samples were albumin depleted using a kit as per the protocol provided by company and the protein concentration was measured using Bradford assay. For 2D gel electrophoresis, 50 µg of follicular fluid proteins was mixed in sample buffer (8M urea, 2% CHAPS, 50mM dithiothreitol, 0.2% biolyte ampholyte, 2M thiourea) and final volume of 125 µl/well in IEF plate was loaded. Precast immobilized pH gradients strips (7cm, pH 3–10) were rehydrated overnight (16 h) in active phase at 20°C in 50 Volts. Later the strips were isoelectric focused as described earlier (Gromova and Celis 2006). The strips were equilibrated in SDS equilibration buffer (6M urea, 57mM Tris-HCl, pH 8.8, 29.3% glycerol and 2% SDS) containing 30 mg/ml DTT for 15 min, followed by incubation in SDS equilibration buffer containing 30mg/ml iodoacetamide for 15 min at room temperature and subsequent wash with electrode buffer (25 mM Tris-base, pH 8.3, 250 mM glycine, 0.1% SDS). The second dimension was carried out using 8–16% precast gels and silver staining was done to detect proteins (Olivier *et al.* 2010). Gels were scanned in gel documentation system and the molecular weight, raw volume and quantity of separated proteins between control and treatment groups were analyzed using software.

Statistical analysis of the data was done as per Snedecor and Cochran (1989). The student T test was used to analyse the various data to check the significance among the mean values between treatment and control groups. The data were

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presented as mean±SEM. P<0.05 was set as significant levels.

The concentration of serum minerals in treatment (Mg, 13.07±0.61 ppm; Cu, 0.73±0.02 ppm and B, 820.38±54.77 ppb) and control (Mg, 12.57±1.06 ppm; Cu 0.68±0.06, ppm and B, 984.5±105.5 ppb) groups during the treatment period was nonsignificant. The concentrations of mineral in follicular fluid was also nonsignificant between treatment (Mg,7.42±3.87 ppm; Cu,2.47±0.16 ppm and B, 4981.00±677.79 ppb) and control (Mg, 5.95±1.67 ppm; Cu, 2.34±0.04 ppm and B, 5169.00±1428 ppb) groups. The buffalo follicular fluid protein analysis showed 320 (raw spots) protein spots in treatment group and 283 (raw spots) protein spots in control groups (Fig. 1A). Out of these, a filtered protein spots of 30 numbers in treatment and 24 numbers in control groups were observed (Fig. 1B). In treatment group, 11 protein spots were differentially expressed with molecular weight ranging from 10.59 to 60.57 kDa (pI3.1 to 9.79); 6 protein spots were differentially expressed in control group with molecular weight ranging from 33.43 to 90.91 kDa (pI 4.29 to 9.75). Scatter plot analysis showed the variation in expression level of proteins with molecular weight of 58.65, 57.5, 55.8 kDa, was increased in treatment as compared to control. Similarly

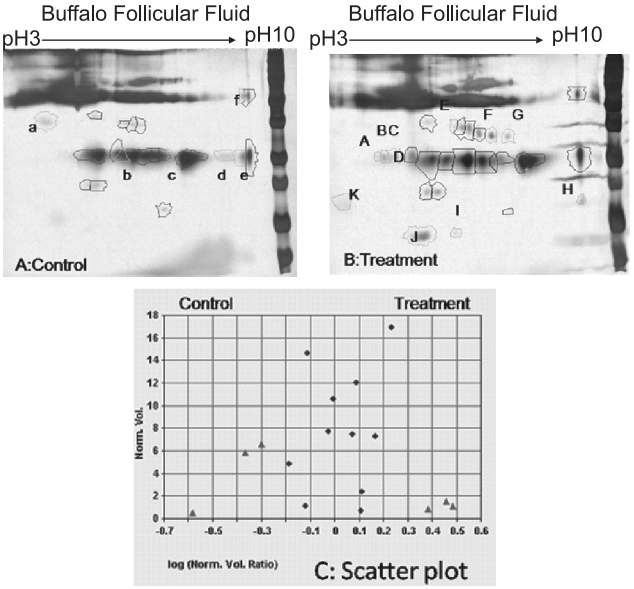


Fig. 1. (A-C) **A&B**. Two dimensional gel electrophoretic analysis of buffalo follicular fluid proteins. The buffaloes were administered with calcium and magnesium during one estrous cycle and follicular fluids were collected from the dominant follicles during estrus. Differentially expressed proteins were observed in control (Spot ID a-f: a- 60.20 pI 4.29, b- 34.05 pI 6.41, c-34.4 pI 7.74, d-34.13 pI 9.27, e-33.43 pI 9.74, f-90.91 pI 9.75) and treatment (Spot ID A-K: A-36.70 pI 4.4, B- 36.18 pI 4.6, C-36.7 pI 4.8, D-36.76 pI 4.97, E-60.57 pI 6.26, F-52.87 pI 7.32, G-51.53 pI 7.75, H-16.27 pI 9.79, I-10.74 pI 6.37, J-10.59 pI 5.46, K-18.02 pI 3.1) groups. Fig. 1 **C.** Scatter plot showing the increased /decreased quantity of certain proteins in control and treatment buffalo follicular fluids of 2DE gels analyzed from Dymension software (Syngene).

the 3 major proteins with molecular weight of 35.12, 33.79, 14.5 kDa, were over expressed in control than the treatment buffalo follicular fluids (Fig. 1C).

As per sex allocation hypothesis, the mother in good condition would be advantaged by producing more of male progeny, whereas mothers in poor condition would be advantaged by producing more of the female progeny (Trivers and Willards 1973). Maternal influences on the uterine environment and sex-selective embryo loss was reported (Grant and Irwin 2005). In the present study, an attempt was made to check the theory of Stolkowski, which hypothesizes that Ca and Mg imbalance in the diet of the female before fertilization affects the sex ratio of progeny by altering mineral and steroid hormone as well as micro environmental conditions of the female reproductive tract. In the present study administration of calcium magnesium borogluconate to buffaloes significantly changed the follicular fluid protein profiles. The observed serum copper level in control group is higher than the values reported earlier (Mudgal *et al*. 2012). Espinoza *et al*. (1991) also observed that external administration of phosphorus to cows increased the certain macro mineral levels in serum and also improved overall reproductive performances. The proposed mechanism from this study is that, administration of calcium and magnesium may change follicular fluid protein profiles, and subsequently it has effect on the oocyte quality as well as surrounding ionic charges towards the type of sperm binding. Probably this could be the first study that documents the influence of calcium and magnesium borogluconate injection on the changes in follicular fluid protein profile in buffaloes. Such modulation of follicular protein profile through diet may be needed in future for clear understanding of the mechanism behind sperm oocyte binding based on ionic charges towards accepting the type or selective population of spermatozoa.

## SUMMARY

An attempt was made in buffaloes (7) with calcium (1.86%) magnesium (5%) borogluconate administration (20 ml subcutaneous route) during one complete estrous cycle length to assess the changes in mineral levels in serum and follicular fluid as well as follicular fluid protein profiles. There was no significant change in serum and follicular fluid mineral (Mg, B, Cu) levels. In the follicular fluid proteins, 11 protein spots in treatment group (10.59 to 60.57 kDa, pI3.1 to 9.79) and 6 protein spots in control group (33.43 to 90.91 kDa, pI 4.29 to 9.75) were differentially expressed. External supplementation of calcium and magnesium changes the protein expressions pattern (increased / decreased quantity) in follicular fluid.

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## REFERENCES


Celik K S, Serbest S, Vurur A, Pala and Daglioglu K. 2003. Experiments to Investigate the Factors That Affect the Rate of Sex Constitution. *Pakistan Journal of Nutrition* **2**(4): 238–41.

Chandraju S, Ashraf Beirami CS and Chidan Kumar. 2011. Effect of calcium and magnesium in identification of baby in high sugar mammals. *Research in Biotechnology* **2**(3): 23–31.

Clint G, Sophie L, Charlotte G, Sheila M G, Jim C and David S Gardner. 2013. Maternal fructose and/or salt intake and reproductive outcome in the rat: effects on growth, fertility, sex ratio, and birth order. *Biology of Reproduction DOI:10.1095/biolreprod.113.109595.*

Espinoza E, McDowell L R, Wilkinson N S, Conrad J H, Martin F G and Williams S N. 1991. Effect of dietary phosphorus level on performance and mineral status of grazing cattle in a warm climate region of central Florida. *Livestock Research for Rural Development* **3**(1):1–4.

Grant V J and Irwin R J. 2005. Follicular fluid steroid levels and subsequent sex of bovine embryos. *Journal of Experimental Zoology* 303A: 1120–25.

Grant V J and Chamley L W. 2010. Can mammalian mothers influence the sex of their offspring peri-conceptually? *Reproduction* **140**(3): 425–33.

Green M P, Spate L D, Parks T E, Kimura K, Murphy C N, Williams J E, Kerley M S, Green J A, Keisler D H and Roberts R M. 2008.Nutritional skewing of conceptus sex in sheep: effects of a maternal diet enriched in rumen-protected polyunsaturated fatty acids (PUFA). *Reproductive Biology and Endocrinology* **6**: 1–11.

Gromova I and Celis J E. Protein Detection in Gels by Silver Staining: A Procedure Compatible with Mass Spectrometry. *Cell Biology.A Laboratory Handbook.* 2006 vol. 4, (Eds) Celis J E, Carter N, Hunter T, Shotton D, Simons K and Small J V. Academic Press, San Diego, CA.

Mudgal V, Gupta V K, Srivastava S and Ganai A A. 2012. Serum concentrations of iron, copper, zinc, manganese and cobalt in anoestrus cattle and buffaloes under farm condition. *International Journal of Livestock Research* **2**(1): 150–59.

Olivier D, Amours, G F, Marlene Fortier, Pierre Leclerc and Robert Sullivan. 2010. Proteomic comparison of detergent extracted sperm proteins from bulls with different fertility indexes. *Society for Reproduction and Fertility* 1470–1626, 1741–7899.

Rosenfeld C S and Roberts R M. 2004. Maternal diet and other factors affecting offspring sex ratio: a review. *Biology of Reproduction* **71**: 1063 70.

Snedecor G W and Cochran W G. *Statistical Methods*. 6th edn. Iowa State University Press, USA. 1989.

Stolkowski J and Lorrain J. 1980. Preconceptional selection of fetal sex. *International Journal of Gynaecology and Obstetrics* **18:** 440–43.

Trivers R L and Willard D E. 1973. Natural selection of parental ability to vary the sex ratio of offspring. *Science* **179**: 90–92.

Vahidi A R and Sheikhha M H. 2007. Comparing the effects of sodium and potassium diet with calcium and magnesium diet on sex ratio of rats offspring. *Pakistan Journal of Nutrition* **6**(1): 44–48.