IMPACT OF INSULIN OR INSULIN LIKE GROWTH FACTOR-I ADMINISTRATION DURING MID-LUTEAL PHASE OF ESTROUS CYCLE ON *IN VIVO* EMBRYO PRODUCTION IN SAHIWAL CATTLE

S.K. SHEETAL[1]*, S. PRASAD[2] AND H.P. GUPTA[3]

Department of Veterinary Gynaecology and Obstetrics
G.B. Pant University of Agriculture and Technology, Pantnagar - 263 145

Received: 03.03.2017 Accepted: 10.03.2017

ABSTRACT

On day 5, 6, 7, and 8 (mid-luteal phase) of estrous cycle, two groups of Sahiwal cattle were administered (S.C.) insulin (n=6) or IGF-I (n=6), whereas, another group was kept as untreated control (n=6). The embryos were collected non-surgically on day 7 of superovulatory estrus. In all the groups, the maximum diameter (mm) of largest follicle and 2nd largest follicle was recorded on day 13 of estrous cycle. The number of ovulations, recovered total embryo/ova, transferable embryos, degenerated embryos and unfertilized ova differed (p>0.05) between groups. Insulin-treated cattle had highest (p>0.05) number of total embryo, transferable embryo and recovered blastocyst. In brief, insulin administration during mid-luteal phase of estrous cycle has the potential to improve *in vivo* embryo production in Sahiwal cattle.

Keywords: Blastocyst, Embryo, IGF-I, Insulin, Sahiwal, Superovulation

INTRODUCTION

Insulin like growth factor-I (IGF-I) of endocrine origin is linked positively to the reproductive performance of cattle (Zhang *et al.,* 2013). Furthermore, insulin administration increases intrafollicular and peripheral IGF-I, which has positive correlation with ovarian follicle growth in cattle (Simpson *et al.,* 1994). In fact, the use of insulin is considered as an approach to improve superovulatory response in cattle through the recruitment of gonadotropin-responsive follicles and by reducing the atresia of follicles. However, the ovarian follicular dynamics of *Bos indicus* cattle is not similar to *B. taurus* cattle which warrants setting up embryo transfer programs for the former breed (Baruselli *et al.,* 2006). Thus, the present study was designed to investigate the impact of insulin and IGF-I administration during mid-luteal phase of estrous cycle on ovarian response, embryo recovery and embryo quality in superovulated Sahiwal cattle.

MATERIALS AND METHODS

Eighteen Sahiwal cattle were equally divided to receive insulin (0.25 IU/kg b wt, S.C.) or IGF-I (10 µg total dose/day, S.C.) on day 5, 6, 7 and 8 of estrous cycle and the third group was kept as untreated control. In all the groups, superovulatory treatment consisting of repeated administration of Follicle Stimulating Hormone (FSH) was started on day 9 after the onset of standing estrus (Folltropin-V, 30 mg twice daily at 12 h interval x 4 day). Along with sixth dose of Folltropin-V, prostaglandin $F_{2\alpha}$ was administered to induce superovulatory estrus. The monitoring of ovarian follicular development (largest and 2nd largest follicle diameter, ovulation rate and number of corpora lutea) on day 9, 11, 13 and 21 of estrous cycle was carried out using B-mode real time ultrasonographic scanning (DIGI 600, PRO VET, SS medical, India). The superovulated cattle were bred twice at 12 h interval through artificial insemination using good quality frozen semen. The embryos were collected non-surgically on day 7 following superovulatory estrus. The embryos and ova were screened under stereozoom microscope

[1]Ph.D. Scholar, [2]Professor and Head, [3]Professor; *sksheetalmuz@gmail.com

(40x). The data were analyzed statistically using Analysis of Variance.

RESULTS AND DISCUSSION

Within groups, the largest follicle and 2nd largest follicle diameter increased (p<0.05) from day 9 to 13 of superovulatory treatment, whereas no difference (p>0.05) was recorded between groups (Table 1). In an earlier study, insulin pre-treatment during summer anestrus increased the diameter of largest follicle in buffalo (Ramoun *et al.*, 2007). Furthermore, the numbers of ovulations and anovulatory follicles in various groups of present study was similar (p>0.05, Table 1). These results were contrary to earlier findings in energy-deprived Brangus heifers in which administration of insulin increased the ovulation rate in response to FSH (Harrison and Randel, 1986).

In the present study, the recovered total embryo/ova, transferable embryo, degenerated embryo and unfertilized ova were similar between groups (p>0.05, Table 1). Nevertheless, the number of total embryo/ova and transferable embryo in insulin-treated Sahiwal cattle was marginally high (p>0.05, Table 1), which was in accordance with an earlier study in goats (Souza *et al.,* 2008).

Table 1: Ovarian response, embryo quality and embryo recovery rate in Sahiwal cattle following administration of Insulin (0.25 IU/kg b wt, S.C.) or IGF-I (10 µg total dose/day, S.C.) on day 5, 6, 7, and 8 (mid-luteal phase) of estrous cycle

Parameters		**Control**, n=6	**Insulin**, n=6	**IGF-I**, n=6
Ovarian response				
Day 9 of estrous cycle	***Largest follilce diameter**, LF, mm	3.91±0.69	4.89±0.46	5.79±0.74
	***2nd LF**, mm	3.41±0.55	3.9±0.29	5.06±0.83
Day 11	***LF**, mm	7.06±0.47	7.39±0.74	8.63±0.35
	***2nd LF**, mm	6.94±0.79	7.07±0.84	7.71±0.45
Day 13	***LF**, mm	10.51±0.18	11.05±0.51	11.67±0.47
	***2nd LF**, mm	10.03±0.6	10.35±0.56	11.07±0.52
Ovulations, n		14.17±1.8	15.17±1.22	14.0±0.73
Anovulations, n		2.33±0.49	1.33±0.49	1.83±0.6
Embryo quality				
Total embryo/ova, n		4.50±1.23	5.83±2.02	5.00±1.93
Transferable embryo, n		3.33±1.89	4.33±1.38	3.17±1.56
Degenerated embryo, n		0.83±0.54	1.17±0.65	1.00±0.45
Unfertilized ova, n		0.33±0.21	0.33±0.21	0.83±0.31
8-16 cell stage embryo, n		0.33±0.21	0.50±0.34	0.50±0.22
Morula, n		2.00±1.13	2.00±1.13	2.00±1.29
Blastocyst, n		1.83±1.14	3.00±0.89	1.67±0.61
Embryo recovery rate				
Total ovulations/corpus luteum, n		85	91	84
Total embryo/ova recovered, n		27	35	30
Embryo recovery rate, %		31.7	38.5	35.7

*p<0.05, between days within a group

The impact of insulin or IGF-I treatment on embryo quality in Sahiwal cattle was absent as the numbers of 8-16 cell stage embryo, morula, blastocyst and unfertilized ova were similar (p>0.05) between groups (Table 1). Nevertheless, among the groups, the blastocysts recovered were marginally higher in insulin-treated and were lowest in IGF-I treated Sahiwal cattle (Table 1). A study in goats exhibited that insulin supplementation increases blastocyst development *in vitro* (Palanisammi *et al.,* 2014). Furthermore, the embryo recovery rate was highest (p>0.05) in insulin-treated (38.5%) Sahiwal cattle followed IGF-I (35.7%) and untreated control (31.7%) groups (Table 1). The present embryo recovery rates are in agreement with an earlier report (Carvalho *et al.,* 2013).

ACKNOWLEDGEMENT

The authors are grateful to Dean, College of Veterinary and Animal Science and Joint Director IDF, Nagla, Pantnagar for providing facilities for conducting this experiment. The authors are also thankful to Director Research G.B.P.U.A.T., Pantnagar, for providing necessary funds.

REFERENCES

Baruselli, P.S., Sa Filho, M.F., Martins, C.M., Nasser, L.F., Nogueira, M.F.G., Barros, C.M. and Bo, G.A. (2006). Superovulation and embryo transfer in Bos indicus cattle. *Theriogenology,* **73**: 77-78.

Carvalho, P., Souza, A., Sartori, R., Hackbart, K., Dresch, A., Vieira, L., Baruselli, P., Guenther, J., Fricke, P. and Shaver, R. (2013). Effects of deep-horn AI on fertilization and embryo production in superovulated cows and heifers. *Theriogenology,* **80**: 1074-1081.

Harrison, L.M. and Rendel, R.D. (1986). Influence of insulin on ovulation rate, lutenizing hormone and progesterone in beef heifers. *J. Anim. Sci.,* **63**: 1228-1235.

Palanisammi, A., Rangasamy, S., Reena, D. and Go, D. (2014). Effect of epidermal growth factor and insulin on *in vitro* maturation of caprine oocytes and pre-implantation embryo developme. *Indian J. Anim. Reprod.,* **35**(1): 25-28.

Simpson, R.B., Chase J.C.C., Spicer, L.J., Vernon, R.K., Hammond, A.C. and Rae, D.O. (1994). Effect of exogenous insulin on plasma and follicular insulin-like growth factor-I, insulin-like growth factor binding protein activity, follicular oestradiol and progesterone, and follicular growth in superovulated Angus and Brahman cows. *J. Reprod. Fertil.,* **102**: 483-492.

Souza, A.L., Galeati, G., Almeida, A.P., Arruda, I.J., Govoni, N., Freitas, V.J.F. and Rondina, D. (2008). Embryo production in superovulated goats treated with insulin before or after mating or by continuous propylene glycol supplementation. *Reprod. Dom. Anim.,* **43**: 218-221.

Zhang, X., Davis, M.E., Moeller, S.J. and Ottobre, J.S. (2013). Effects of selection for blood serum IGF-I concentration on reproductive performance of female Angus beef cattle. *J. Anim. Sci.,* **91**(9): 4104-4015.