OVARIAN STEROIDAL PROFILE AND FERTILITY TO INSULIN AND GnRH ADMINISTRATION IN POSTPARTUM ANESTRUS BUFFALOES

GUPTA V. K. †, S. N. SHUKLA, M. S. THAKUR AND R. G. AGRAWAL

Department of Animal Reproduction, Gynaecology & Obstetrics, College of Veterinary Science & A.H., MPPCVV, Jabalpur-482001, MP, INDIA

Received : 16.11.2010

ABSTRACT

Accepted : 14.09.2011

To study the effect of exogenous administration of insulin on ovarian and fertility response in anestrus buffaloes, 18 true anestrus buffaloes were selected and equally divided into 3- groups (n=6). Animals of group-I (G1) received subcutaneous injection of highly purified long acting bovine insulin at the dose of 0.25 IU kg^{-1} bodyweight, once daily for 5 consecutive days. Animals of group-II (G2) received an intramuscular injection of 20 µg buserelin acetate where as animals of group-III (control) received an intramuscular injection of 2.5 ml sterile saline. In response to the treatment, 50 and 33.33% animals were induced in estrus with in 25 ± 0.94 and 9.5±3.18 days from start of treatment, respectively in G1 and G2; however, no animal was induced in estrus in control group. Conception rate at induced estrus was 100% in both the treatment groups. The mean serum progesterone (P_4) concentration was less than 1ng/ml on day before start of treatment, day 7th and 14th following start of treatment in all the experimental animals. The basal level of P_4 concentration (ng/ml) at induced estrus (0.44 ±0.25 and 0.19 0.04) increased (4.46 ± 0.22 and 4.47 ± 0.58) significantly ($p<0.05$) at day 10 post estrus, respectively, in G1 and G2. No significant difference in serum estradiol-17b(E_2) concentration was recorded on day before start of treatment, day 7th, 14th following start of treatment and day 10th post estrus either within or between the treatment groups. It may be concluded that exogenous administration of insulin stimulates the ovarian and fertility response in anestrous buffaloes.

Key words: Insulin, anoestrus, fertility, Buffalo, GnRH

Anestrus is one of the most important reproductive problems in buffaloes. It is a functional disorder of ovary affecting production potential in the form of huge economic losses. Higher incidence of anestrus due to inactive ovaries has been reported in buffaloes (Agarwal and Tomer, 1998; Singh *et al.* 2000). Several hormonal and non hormonal therapies have been tried by several workers for the management of anestrus.

Application of insulin to modulate reproduction in livestock is recently focused. Recombinant growth hormone and positive energy balance altered the ovarian activities with increase in circulatory insulin like growth factors (IGF-1) and insulin as well intra follicular IGF-1 concentration (Downing *et al.*, 1995). Insulin enhances steroidogenesis and folliculogenesis by modulating follicular growth factor system and steroidal hormones (Simpson *et al.*, 1994). It also regulates gonadotropin releasing hormone (GnRH) / pulsatile luteinizing hormone (LH) secretion (Tanaka *et al.* 2000).

First time, the exogenous insulin was reported as an effective measure for management of anoestrus in cattle (Shukla *et al.*, 2005a & b) and goat (Sarath *et al.*, 2008) since insulin can be a factor regulating growth, maturation and ovulation of dominant follicle. But, such informations are lacking in buffaloes. Insulin is cheap and easily available to be useful in management of anestrus in buffalo. Therefore, the present experiment was designed to study the ovarian functions and fertility

†-Teaching Associate (Corresponding author)
Present Address: - Department of Animal Reproduction Gynaecolgy &Obst., College of Veterinary science &A.H., MHOW-453446, MPPCVV (M.P.)
Email. I.D. - dr_vkvet23@rediffmail.com

response using insulin in anoestrus buffaloes.

MATERIAL AND METHODS

The present experiment was conducted at Composite Livestock Farm, Adhartal, Jabalpur (MP). Eighteen apparently healthy, non suckling lactating, acyclic buffalo cows (>90 days post-partum), weighing approximately 450-500 kgs and 4-8 years in age were used for the study. The experimental period extended from November to March when the relative humidity and ambient temperature was 70-80% and 25-30°C, respectively. The buffaloes were stall-fed and housed in concrete sheds with standard managemental norms of Livestock Farm. They were fed on a standard diet calculated to meet both their maintenance and milk production requirements.

The confirmation of anestrus was made on the basis of history, gynaecological examination of genitalia twice at weekly interval and serum progesterone assay. Animals having clinically smooth ovaries (without palpable corpus luteum and follicle) in both the rectal examinations but normal developed genitalia and serum progesterone concentration less than 1ng/ml were selected and divided equally into 3-groups, (n=6) animals. Animals of group-I (G1) received subcutaneous injection of long acting bovine insulin (Vinsulin; M.J. Biopharma Pvt. Ltd, Mumbai, India) at the dose @ 0.25 IU kg^{-1} bodyweight, once daily for 5- consecutive days. Animals of group-II (G2) received an intramuscular injection of 20 µg buserelin acetate (Receptal VET, Intervet). Animals of group-III (G3) served as untreated control.

All the animals were observed for sign of estrus and by buffalo teaser bull twice daily at morning and evening through out the study period. The estrus induction rate was determined based on the results of visual observations for estrus signs, teasing results and changes in serum progesterone and estradiol concentrations for each group. All the animals were examined per rectally at 7-days interval after treatment to monitor the ovarian and uterine changes. Buffaloes were bred by natural service at induced estrus using fertile bull. Ovulatory response was studied by per rectal examination and serum progesterone level at day 10th post estrus for the presence of corpus luteum in the ovary. Pregnancy diagnosis was carried out after 60 days of breeding. The time taken for onset of estrus following withdrawal of treatment, occurrence of ovulation and fertility at induced estrus was calculated and analyzed.

The blood samples were collected aseptically by jugular vein puncture from all the animals on day-0 (at the start of treatment); -7 and 14 following start of treatment, at estrus and on day 10th post estrus. Then serum was harvested and stored at -20°C until hormone assay.

The quantitative determination of progesterone and estradiol-17α concentrations in serum was made by Enzyme-Linked-Immuno-Sorbent-Assay (ELISA) using kits supplied by Biotron Diagnostics Inc, Hemet, California, USA. The Sensitivity, intra-assay variation (CV %), inter-assay variation (CV %) and Accuracy (%) of estradiol and progesterone kits were 10 pg/ml and 0.3 ng/ml, 5.74 and 15.86, 4.43 and 8.61, 91.21 and 94, respectively.

The data were analyzed statistically for the mean and standard error. Comparison of estradiol-17α and progesterone levels at different days was done using CRD at 5% significance level (Snedecor and Cocharan, 1994).

RESULTS AND DISCUSSION

The induction of estrus was higher in insulin (50% -G1) as compared to GnRH (33.33%-G2) treated animals, however, no animal showed in estrus in control. The time taken for onset of estrus was shorter (9.5±3.18) in GnRH as compared to insulin (250.94 days) treated animals. The ovulation and conception rate at induced estrus was 100% in animals of both the treatment groups.

The beneficial effect of insulin on induction of estrus, resumption of ovarian cyclicity and fertility in anestrus buffaloes are comparable to the findings of Shukla *et. al.* (2005a & b) and Ramoun *et al.* (2007) which may be due to its effects on folliculogenesis and

steroidogenesis. The induction of estrus was higher in insulin alone (85.7%) as compared to GnRH (57.14%), and untreated control (14.3%) anestrus cows. The onset of estrus interval (hrs) following start of treatment was shorter in insulin (180±42.82), GnRH (186±45.29) as compared to control (240±156.15) (Shukla *et. al.*, 2005a & b). Ramoun *et al.* (2007) reported pre-treatment with insulin for 3 days before GnRH injection increases the diameter of the dominant follicle and therefore the estrous induction rate of acyclic buffaloes.

In vitro studies have also supported such findings where insulin and IGF-1 have been demonstrated as important regulators of folliculogenesis and steroidogenesis (Gong *et al.*, 1994, Stewart *et al.*, 1995) through growth and proliferation of granulosa, theca and luteal cells of the ovary (Stewart *et al.*, 1995). Structural and functional relationship of IGF-1 and insulin suggested the role of insulin in synthesis of IGF-1 which is a potent ovarian growth factor and acts through autocrine and paracrine manner (Stewart *et. al.*, 1995). It increases the number of granulosa cells present in follicles, the source of estrogen and thus enhances the production of estradiol -17α. This may be the reason for increased level of estradiol concentration and pronounced uterine tonicity following insulin administration observed in the present study.

Better conception was also reported on insulin administration during diestrus in repeat breeding cattle (Selvaraju *et al.* 2002; Kharche *et al.*, 2003). This may be due to luteotrophic effect of insulin in ovary. Insulin may stimulate IGF-I secretion which is found to be a potent ovarian and uterine growth factor mediating the reproductive function (Pawshe, 1998). In vitro studies revealed better early embryonic development with use of insulin and IGF-I (Totey *et al.*, 1996). Insulin enhances growth and proliferation of granulosa, theca and luteal cells present in the ovary, thus enhances folliculogenesis (Simpson *et al.*, 1994). Insulin along with IGF-I enhances growth and proliferation of theca cells leading to production of progesterone (Stewart *et al.*, 1995). Insulin enhanced pulsatile LH secretion acting through CNS a pre-requisite for maturation of dominant follicle has been reported (Tanaka *et al.*, 2000).

GnRH and its analogue has been used for augmentation of fertility in anestrus buffaloes (Pattabiraman *et al.*,1986) reported that out of 15 anestrus buffaloes treated with a single intra-muscular injection of 5 ml receptal, 8 animals showed signs of estrus within 13 to 22 days, 6 ovulated and 3 animals conceived. Markandeya and Patil (2003) reported that post-partum anestrus buffaloes treated with GnRH (Receptal) therapy showed 66.67 % induction of estrus within 4 to 7 days of treatment. GnRH induces LH surge and ovulation when given in post-partum dairy animals (Foster *et al.*, 1980). This would explain the findings of higher ovulation rate obtained in the present study.

The serum progesterone concentration a day before start of treatment ranged between 0.43 ± 0.09 to 0.51 ± 0.10 ng/ml i.e. less than 1ng/ml in all the experimental animals, confirming the anestrus state of animals and the results of rectal palpation. Similar observations in anestrus cattle have been reported by Shukla *et al.*, (2005b). The mean serum progesterone concentration ranged from 0.37±0.11 to 0.66±0.23 at day 7th and 0.56+0.07 to 0.73+0.13 ng/ml at day 14 following start of treatment. It may be due to the absence of corpus luteum or lack of luteal cells in anestrous ovaries or progesterone produced by theca cells may be aromatized into estradiol-17α (Stewart *et al.*, 1995). Serum progesterone concentration was fluctuated at basal level (0.19 ± 0.04 to 0.44 ±0.25) at estrus, increased thereafter and reached to its highest level (4.27 0.35 to 4.47 ± 0.58 ng/ml) on day 10th post estrus in both the treatment groups. It indicates presence of functional corpus luteum and confirming the results of rectal palpation. It may also suggest the luteotropic effect of insulin responsible for 100% (3/3) conception. Similar findings were also reported by Shukla *et al.* (2005b) in cattle.

The mean serum estradiol-17α (E_2) concentration day before treatment ranged between 12.03 ± 1.53 to 14.59 ±0.84 pg/ml in all the experimental animals. Our results are in consistent with the findings of Batra and Pandey (1982) and confirming the results of rectal palpation and anestrous state of all the experimental animals. The mean serum estradiol-17α concentration

ranged from 11.56 ± 1.18 to 13.93 ± 0.96 at day-7, 12.13 ± 0.93 to 14.55 ± 0.83 pg/ml at day-14 following start of treatment indicating no significant difference in neither within nor between the treatment groups and control on same day of the sampling. The serum estradiol-17α concentration significantly ($P< 0.05$) increased at estrus ranging from 45.28 ± 3.35 to 47.69 ±3.92 pg/ml in all the treatment groups as compared to day before treatment on day 7th, 14th following start of treatment and day 10th post estrus. Similar findings have been reported by Batra and Pandey (1982). This may be due to aromatization of progesterone into estradiol by theca cells. After estrus, the estradiol- 17α concentration was (13.08 ± 1.32 to 14.24 ± 1.05 pg/ml) significantly ($p<0.05$) declined on day 10th post estrus in all the treated animals similar to findings of Batra and Pandey (1982).

Structural and functional relationship of IGF-1 and insulin suggested the role of insulin in synthesis of IGF-1 which is a potent ovarian growth factor and acts through autocrine and paracrine manner (Stewart *et. al.,* 1995) which enhances the growth of granulosa cells and thus the production of estradiol -17α. This may be the reason of increased level of estradiol concentration and pronounced uterine tonicity following insulin administration observed in the present study.

It may be concluded that administration of insulin has beneficial effects on ovarian and fertility response in anestrous buffaloes. It is also cheap and easily available thus can be used for management of post partum anoestrus in buffaloes. However, the study needs to be repeated in a sizable number of animals before making any recommendation for use at field level.

REFERENCES

Agarwal, S.K. and O.S. Tomer (1998). Reproductive Technologies in Buffalo. Publication of IVRI, Izatnagar, Bareilly, U.P., India

Batra, S.K. and R. S. Pandey (1982) Luteinizing hormone and oestradiol - 17b in blood plasma and milk during the oestrous cycle and early pregnancy in Murrah buffaloes. *Anim. Reprod. Sci.,* **5**: 147-157.

Downing, J.A., J. Jossand and R.J. Scaramuzzi (1995). Ovulation rate and conception of gonadotropins and metabolic hormones in ewes infused with glucose during the late luteal phase of the estrous cycle. *J. Endocrinology.*, **146**: 403-410.

Foster, J.P., G. E. Lamming and A. R. Peters (1980). Short term relationships between plasma LH, FSH and progesteron concentrations in post-partum dairy cows and the effect of GnRH injection. *J. Reprod. Fertil.,* **59**: 321-327.

Gong, J.B., D. McBride, T.A. Bramley and R. Webb (1994). Effects of recombinant bovine somatotropin insulin - like growths factors-I and insulin on bovine granulosa cell steroidogenesis in vitro. *J. Reprod. Fertil.*, **110** : 611-622.

Kharche, S.D., A. C. Majumdar and S. K. Srivastava (2003). Effect of insulin administration at oestrus on fertility of crossbred cattle. *Indian J. Anim. Sci.*, **73**: 888-889.

Ramoun, A.A., A.D. Khalid, T. Osman, B. Samy, A. Darwish, B. Aly, M. Karen, A. Magdy and H. Gamal (2007). Effect of pretreatment with insulin on the response of buffaloes with inactive ovaries to gonadotrophin-releasing hormone agonist treatment in summer. *Reprod., Fertil. and Dev.*, 2007, **19**: 351–355

Ricoy, E.F., C. Aeua, R.M. Rincon, D.F. Cortes, R. Bauelos-Valenzuela and C. F. Arechiga (2000). True oestrus determination through evaluation of serum progesterone levels at the time of insemination of dairy cows from semi intensive dairies in north-central Mexico. *J. of Anim. Sci.,* **79**: 228

Sarath, T., S. Mehrotra , S.K. Agarwal, V.P. Varshney , M. Hoque, U. Shankar and S. K. Singh (2008). Effect of insulin administration on ovarian function and estrus induction in acyclic goats. *Anim. Reprod. Sci.,* **108(1-2)**:216-225

Selvaraju, S., S.K. Agarwal, S.D. Karche, S.K. Srivastava, A.C. Majumdar and U. Shanker (2002).

Fertility responses and hormonal profiles in repeat breeding cows treated with insulin. *Anim. Reprod. Sci.*, **73**: 141-149.

Shukla, S.N., S. K. Agarwal, U. Shanker, V.P. Varshney and A.C. Majumdar (2005a). Ovarian function and restoration of fertility using insulin in acyclic dairy Cattle. *Indian. J. Anim. Sci.*, **75 (10)**:1135-1139.

Shukla, S.N., S. K. Agarwal, U. Shanker, V.P. Varshney and A.C. Majumdar (2005b). Modulation of ovarian response in anoestrus cattle treated with insulin alone and in combination with GnRH. *Indian J. Anim. Reprod.*, **26 (2)**:159-164.

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

Singh J., A.S. Nanda and G.P. Adams (2000) The reproductive pattern and efficiency of female buffaloes. *Anim. Reprod. Sci.*, **61:** 593-604.

Snedecor, G.W. and W.G. Cocharan (1994). Statistical Methods, 6th edn. Oxford and IBH, New Delhi.

Stewart, R.E., L.J. Spicer, T.D. Hamilton and B.E. Keefer (1995). Effects of insulin like growth factor-I and insulin on proliferation and on basal luteinizing hormone induced steroidogenesis of bovine thecal cells: involvement of glucose and receptors for insulin-like growth factor-I and luteinizing hormone. *J. Anim. Sci.*, **73**: 3719-3731.

Tanaka, T., S. Nagalani, D.C. Bucholtz, S. Onkura, H. Tsukamura, K. Maeda and D. L. Foster, (2000). Central action of insulin regulates pulsatile leuteinizing hormone secretion in the diabetic sheep model. *Biol. of Reprod.*, **62**: 1256-1261.

ISSAR AWARD

Dr. N.C. Sharma Award

- The award is for the best paper published in the Indian Journal of Animal reproduction during the year preceeding the year of award.
- The first author should be a life member of ISSAR.
- The award in the form of a memento/trophy will be given to the first author and certificates to all authors during the inaugural function of the Annual convention of the society.