ROLE OF hCG IN FOLLICULAR, LUTEAL CHARACTERISTICS, SERUM PROGESTERONE AND ESTRADIOL CONCENTRATION IN BUFFALOES*

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ABSTRACT

The present study was conducted with the objective to improve conception rate through exogenous administration of hCG (Chorulon®, Intervet India Pvt Ltd., India) on day 0 of estrous cycle in Murrah buffaloes. Estrus was induced using 500 µg Cloprostenol sodium im. and they were bred naturally during the observed estrus. The buffaloes in treatment group (n=10) had been given hCG @ 1500 I.U. im. and control group (n=10), PBS @ 5ml im. on day 0 of estrous cycle. After 35 to 45 days of breeding the buffaloes were scanned through ultrasonography for the pregnancy diagnosis. Development of ovulatory follicle and the dominant follicle of 1st wave of treatment and control group was recorded. There were no significant differences in the follicular diameter between pregnant and non-pregnant buffaloes of treatment and control group. Development of corpus luteum (mm^2) was significantly ($P < 0.01$) greater on day 6 to 20 of estrous cycle in treatment group, while only on day 18 and 20 ($P < 0.01$) in control group. Serum progesterone concentration was significantly ($P < 0.01$) higher on days 18 and 20 of estrous cycle in pregnant than non-pregnant buffaloes. The concentration of estradiol 17â found significantly ($P < 0.01$) lower in pregnant buffaloes on days 1, 2, 6, 10, 14, 18 and 20 of estrous cycle than non-pregnant buffaloes of treatment group .Thus it was concluded that follicular diameter of treated pregnant buffaloes were greater than the control pregnant buffaloes, corpus luteum of pregnant buffaloes were greater than non-pregnant, serum progesterone concentration was higher in pregnant buffaloes than the non-pregnant and the serum estradiol 17α was lower in pregnant than non-pregnant buffaloes.

Key words: hCG, Follicular development, Corpus luteum, Progesterone, Estradiol, Buffalo

INTRODUCTION

Buffalo is one of the most important domestic ruminants centered mostly in tropical and subtropical regions of the world. Buffalo is considered as the backbone of Indian farmer's economy and dairy industry. One factor that contributes to the loss of embryos is deficiency of serum progesterone, which might be due to insufficiency of corpus luteum. In order to reduce the incidence of early embryonic mortality due to luteal insufficiency,several corrective measures have been suggested. These include the use of serum progesterone supplementation from an exogenous source (Flint *et al.,* 1991), uterine infusion of embryonic vesicles or products (Betteridge *et al.,* 1980) or injection of luteotropic agent, such as hCG (Nephew *et al.,* 1994) or hypothalamic hormone like gonadotropin releasing hormone (Nakao *et al.,* 1983). However, these measures have shown inconsistent results regarding their effect on endogenous serum progesterone level and pregnancy rates.

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Human chorionic gonadotropin has been reported to stimulate blastocyst expansion, and larger blastocysts secrete more interferon (IFN), (Nephew *et al.,* 1994), which by down–regulation of serum estradiol and oxytocin receptor suppresses $PGF_{2\alpha}$ release more effectively. Consequently, the luteolytic mechanism is either blocked or delayed and this gives blastocysts more time to establish, hCG may also enhance conception rate significantly when used post-insemination.

Reproductive problems are more among buffalo than cattle, hence buffalo is considered difficult breeder primarily because of its inherent susceptibility with environmental stress, which causes anestrus and subestrus (Singh *et al.,* 1988). These conditions are responsible for prolonged inter-calving period, resulting in great economic losses to the dairy industry. Subestrus or silent estrus contributes the largest factor responsible for the poor reproductive efficiency in the buffaloes.

Based on the earlier research works, the present study was designed to ascertain the role of hCG in advancing follicular development, luteal characteristics, serum progesterone and estradiol concentration in Murrah buffaloes.

MATERIAL AND METHODS

Twenty normal cyclic buffaloes were selected on the basis of records and through per-rectal examination of the genital organs. The experimental buffaloes, aged 4-7 years were divided into two groups; treatment (n=10) and control (n=10). The experimental buffaloes were given broad spectrum anthelmintics (Fentas® @ 5-7.5 mg/kg body weight single dose), 15 days prior to induction of estrus. Buffaloes having (Corpus luteum) CL, were injected 500 µg Cloprostenol sodium (Cyclix®, 2ml im, Intervet India Pvt. Ltd., India). The estrus detection was done in the morning and evening by close observation of external signs. Estrus is further confirmed by using transrectal probe and scanning the size of pre-ovulatory follicle (9-10 mm in diameter). Animals were bred naturally 12 hrs after the beginning of estrus with fertile bull.

hCG 1500 I.U. im. (**Chorulon®**, Intervet India Pvt. Ltd., India) was given on day 0 in treatment group and 5 ml PBS im on day 0 of estrous cycle in control group. Blood samples were taken by jugular venipuncture on days 0, 1, 2, 6, 10, 14, 16, 18 and 20. About 4-5 ml blood without anticoagulant was collected in sterilized glass tubes and kept at room temperature as a slant for 6-8 hours for separation of blood serum which were centrifuged at 3000 rpm for 15 minute at room temperature and was transferred into sterilized serum vials stored at -20°C till analysis.

The data obtained in the present study was analyzed statistically and subjected to the test of significance as per the methods described by Snedecor and Cochran (1967). The results were expressed in term of mean and their standard errors and were compared using Independent sample t-test.

RESULTS AND DISCUSSION

The maximum and minimum diameter (mm) of dominant follicle recorded was 11.44 ± 0.87 mm and 4.73 ± 1.97 mm on day 10 and 2, respectively, in pregnant buffaloes, whereas, the corresponding values of dominant follicle of non-pregnant buffaloes were 12.51 ± 0.42 mm and 4.33 ± 1.59 mm . The maximum diameter of ovulatory follicle recorded was 11.13 ± 1.42 mm and 10.91 ± 1.58 mm on day 1 in pregnant and non-pregnant buffaloes, respectively. In control groups, the maximum and minimum diameter (mm) of 1st wave dominant follicle recorded was 10.84 ± 0.43 mm and 4.73 ± 0.28 mm on days 10 and 20, respectively, in pregnant buffaloes while the corresponding values of 1st wave dominant follicle recorded in non-pregnant buffaloes were 11.11 ± 0.36 mm and 4.96 ± 0.17 mm. The maximum diameter of ovulatory follicle in pregnant and non-pregnant control buffaloes were found to be 11.97 ± 1.89 mm and 11.84 ± 1.78 mm, respectively, on day 2 of estrous cycle. There were no significant differences in follicular development of ovulatory and 1st wave dominant follicles in pregnant and non-pregnant buffaloes of treatment and control group.

The increase in the size of the 1st wave dominant follicle of treatment group was due to hCG in the present

study. These findings are well supported by Kelidari *et al.* (2010). In the present work the injection of hCG cause early ovulation of preovulatory follicle in treatment group as compared to control because hCG has LH like activity and the pre-ovulatory LH surge is necessary for ovulation. The scanning of 1st wave dominant follicle, irrespective of 2 or 3 waves cycle, has been done and there was regression of 1st wave dominant follicle by day 20 of estrous cycle. The 1st wave lasts till day 24 and the persistence of 1st dominant follicle was 20.67 ± 1.18 days of 2 wave cycle and 17.9 ± 3.47 days of 3 wave cycle as reported by Baruselli *et al.* (1997).

The effect of hCG on the development of corpus luteum of pregnant and non-pregnant buffaloes of treatment group revealed that the maximum and minimum area (mm^2) of corpus luteum found was 143.19 ± 1.544 mm^2 and 41.470 ± 0.884 mm^2 on day 20 and 6 of estrous cycle, respectively in pregnant buffaloes, whereas, in non-pregnant buffaloes the corresponding values of corpus luteum were 81.812 ± 0.441 mm^2 and 32.966 ± 1.081 mm^2 on day 16 and 6 of estrous cycle, respectively. The effect of hCG on corpus luteum area was found significant ($P < 0.01$) on days 6, 10,14,16,18 and 20 of estrous cycle in pregnant and non-pregnant buffaloes.There were significant differences in the development of corpus luteum among the pregnant and non-pregnant buffaloes of control group on day 18 and 20 ($P < 0.01$) of estrous cycle. The maximum and minimum values of mean area were 120.951 ± 4.794 mm^2 and 37.678 ± 1.073 mm^2 in pregnant buffaloes on day 20 and 6, respectively, whereas, the maximum and minimum values were 86.818 ± 0.815 mm^2 and 35.218 ± 0.791 mm^2, respectively on day 16 and 6 of estrous cycle in non-pregnant buffaloes.

The increase in size of corpus luteum was due to the positive effect of hCG with its LH like activity, which might have provided luteotropic stimulation to corpus luteum and increased its area. This finding agreed with the study of Farin *et al.* (1988) that hCG treatment increases luteal weight and diameter in sheep and cattle. The luteal stimulation due to hCG may either be in the form of conversion of small luteal cells to large luteal cells (Farin *et al.*, 1988; Nephew *et al.*, 1994) or may even be ascribed to an increase in the size of large luteal cells (Fitz *et al.*, 1982).

There were significant differences ($P < 0.01$) in serum progesterone profile of pregnant buffaloes of treatment group on day 18 and 20 of estrous cycle due to exogenous hCG administration (Table 3). The highest and lowest concentration of serum progesterone in pregnant treated buffaloes were 6.338 ± 0.140 ng/ml and 0.601 ± 0.027 ng/ml on day 20 and 0, whereas, in non-pregnant treated buffaloes the corresponding values were 3.802 ± 0.181 ng/ml and 0.541 ± 0.049 ng/ml on day 16 and 0, of estrous cycle respectively.

The significant ($P < 0.01$) variation in serum progesterone profile in pregnant buffaloes of control group was only on day 18 and 20 of estrous cycle. The highest and lowest concentration of serum progesterone in pregnant buffaloes were 5.910 ± 0.139 ng/ml and 0.488 ± 0.014 ng/ml on day 20 and 0 of estrous cycle, respectively, whereas, in non-pregnant buffaloes the corresponding values were 4.219 ± 0.038 ng/ml and 0.473 ± 0.026 ng/ml on day 16 and 0, respectively.

The increase in the concentration of serum progesterone observed due to hCG in the present study is consistent with the reports of Rettmer (1991), which revealed that serum progesterone concentration increased 24 hrs after treatment with hCG and remained elevated above controls until onset of luteolysis. Nishigai *et al.* (1998) stated that serum progesterone level increased 3 hrs after the hCG administration.

The levels of estradiol in pregnant buffaloes of treatment group were significantly different on days 1, 2, 6, 10, 14, 18 and 20 ($P < 0.01$) of estrous cycle due to exogenous hCG administration. The maximum and minimum concentration of estradiol in pregnant buffaloes were 18.482 ± 0.241 pg/ml and 3.526 ± 0.052 pg/ml on days 0 and 20 of estrous cycle, respectively, whereas, in non-pregnant buffaloes the corresponding values were 25.270 ± 0.239 pg/ml and 5.250 ± 0.024 pg/ml on days 20 and 2 of estrous cycle, respectively.

The effect of hCG on the concentration of serum estradiol 17â in control group revealed that the maximum

and minimum values in pregnant buffaloes were 17.670 ± 0.154 pg/ml and 4.188 ± 0.078 pg/ml on day 0 and 16 of estrous cycle, respectively, while in non-pregnant buffaloes the corresponding values were 28.306 ± 1.277 pg/ml and 5.590 ± 0.349 pg/ml on days 20 and 2, variations respectively.

There were significant ($P < 0.01$) variation in the mean concentration of serum estradiol in between pregnant and non-pregnant buffaloes of control group on days 10, 14, 18 and 20 of estrous cycle.

The concentration of estradiol in non-pregnant control buffaloes on day 0 was 17.242 ± 1.196 pg/ml, which is in agreement with the finding of corresponding value of Bachalaus *et al.* (1980) was 17.8 pg/ml . The value of estradiol on day 10 of the present study of non-pregnant buffalo of control group was 20.176 ± 0.239 pg/ml which are similar with the reports of Agarwal and Tomar (2003) that day 10 after estrus the value was around 20 pg/ml. They further reported that in non-pregnant buffaloes the estradiol value fluctuated at a lower level throughout the luteal phase of cycle except on day 4 and 10, where the minor peaks of 10 pg/ml and 20 pg/ml were found, these pea ks were due to the waves of follicular growth.

Present results are in agreement with the finding of Bennett *et al.* (1991) that exogenous administration of hCG during early luteal phase did not affect subsequent luteolysis response of corpus luteum to $PGF_{2\alpha}$ but did significantly reduce the estradiol level.

REFERENCES

Agarwal, S. K. and Tomer, O. S. (2003). Reproductive Technologies in Buffalo. 2nd ed. Indian Veterinary Research Institute, Izatnagar, U.P.127.

Bachlaus, N. K., Arora, R. C., Prasad, A and Pandey, R. S. (1980). Effect of prostaglandin $F_2\alpha$ *Bubalus bubalis*). *Theriogenology,* **13**(4): 297-303.

Baruseli, P. S., Mucciolo, R. G., Visintin, J. A., Viana, W. G., Arruda, R. P., Maduriera, E. H., Oliveira, C. A. and Molero Filho, J. R. (1997). Ovarian follicular dynamics during the oestrus cycle in buffalo. *Theriogenology,* **47**: 1531-1547.

Bennett , G. L. and Leymaster, K. A. (1990). Genetic implications of a simulation model of litter size in swine based on ovulation rate, potential embryonic viability and uterine capacity: I. Genetic theory. *J. Anim. Sci.* **68**:969-979.

Betteridge, K. J., Eaglesome, M. D., Randaland, G. C. B. and Mitchell, D. (1980). Collecton, description and transfer of embryo from cattle. *J. Reprod. Fertil.* **59**: 205-216.

Farin, C. E., Moeller, C. L., Mayan, H., Gamboni, F., Sawyer, H. R. and Niswender, G. D. (1988). Effect of leuteinizing hormone and chorionic gonadotropin on cell populations in the ovine corpus luteum. *Biol. Reprod.,* **38**: 413-421.

Fitz, T. A., Mayan, M. H., Sawer, H. R. and Niswender, G. D. (1982). Characterization of two steroidogenic cell types in the ovine corpus luteum. *Biol. Reprod.*, **27**. pp.703-711.

Flint, A. P. F., Parkinson, T. J., Stewart, H. J., Valet, J. L., and Lamming, G. E. (1991). Molecular biology of trophoblast interferons and studies of their effects in *vivo. J. Reprod., Fertil.*, **43**: 13-25.

Kelidari, H. R., Souri, M., Shabankareh, H. K. and Hashemi, S. B. (2010). Repeated administration of hCG on follicular and Luteal characteristics and serum progesterone concentrations in eCG-Superovulated does. *Small Rumin. Res.,* **90** ,1-3, p 95-100.

Nakao, T., Narita, S., Tanaka, K., Horn, H., Shirakawa, J., Noshiro, H., Saga, N., Tsunoda, H. and Kawata, K. (1983). Improvement in first service pregnancy rate in cows with gonadotropin- releasing hormone analogue. *Theriogenology,* **20**: 111-119.

Nephew, K. P., Cardenas, H., McClure, K. E., Ott, T. L., Bazer, F. W. and Pope, W. F. (1994). Effects of administration of human chorionic gonadotropin or progesterone before maternal recognition of

pregnancy on blastocyst development and pregnancy in sheep. *J. Anim. Sci.,* **72**: 453-458.

Nishigai, M., Kamomae, H., Tanaka, T. and Kaneda, Y. (1998). Pregnancy rate and blood progesterone concentration on the previous day and the day of frozen embryo transfer in parous recipient cows of Japanese Black. *J. Reprod. Dev.,* **44**: 413-419.

Rettmer, I. (1991). Administration of a GnRH analog agonist (fertirelin acetate) during the mid-luteal phase of the estrous cycle: Effects on pregnancy rates, hormonal profiles and ovarian follicular and luteal dynamics. MS Thesis, Kansas State Univ. Manhattan.

Singh, G., Dhaliwal, G. S., Sharma, R. D., Biswas, R. K. and Hemeida, Janakiraman. (1988). Treatment of summer anestrus buffalo (*Bubalus bubalis*) with the progesterone releasing intravaginal device plus pregnant mare serum gonadotropin. *Theriogenology,* **29** (5): 1201-1206.

Snedecor, G. W. and Cochran, W. G. (1967). Statistical Methods. 6th ed., Oxford and IBH. The Iowa State University Press, Ames, Iowa , U.S.A.

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