Global Veterinaria 4 (4): 337-342, 2010
ISSN 1992-6197

Assessment of Superovulatory Response Using Hormonal Profile in Buffalo (*Bubalus bubalis*)

Bassiouni Heleil, Ismail El-Kon and Yasser El Deeb

Department of Theriogenology, Faculty of Veterinary Medicine,
Kafr-Elsheikh University, Kafr-Elsheikh, Egypt

Abstract: This study was designed to ascertain the relationship between serum hormonal profiles and superovulatory response in buffalo (Number of corpora lutea and recovered embryos). A total of 22 buffaloes were used in the present study. Animals were randomly allocated to two groups, Group 1 (10 buffaloes) was injected with normal saline (Control group) group 2 (12 animals) was superovulated by injection of 3000 IU PMSG at mid luteal phase of the estrous cycle (Superovulated group).Both treated and control groups were synchronized by injection of prostaglandin F2á (double intramuscular injections, 11 day apart). Blood samples were collected daily from all animals for determination of serum progesterone and estradiol levels by Radio immunoassay (RIA). Superovulatory response was monitored daily by using transrectal ultrasonography. In superovulated group, results were classified according to the number of corpora lutea (CL) into high response group (HRG) ≥ 3CL (4.85±0.33 CL) and low response group (LRG) <3CL (1.8±0.14 CL). There was significant ($P<0.05$) difference between both superovulated groups in the superovulatory response. Progesterone concentrations on the day of superovulatory treatment were significantly ($P<0.05$) high in HRG (2.3 ±13 ng/ml) than control (1.2±0.12 ng/ml) and LRG (1.28±0.08 ng/ml). In both groups (HRG and LRG), progesterone levels on the day of PMSG injection and on the day of flushing were positively correlated with the superovulatory response. Progesterone concentration on the flushing day in HRG was nearly double and 4 times than the corresponding values in LRG and control one, respectively. Serum estradiol profile did not differ among the control and experimental groups at the start of treatment. Moreover, it was not correlated with the subsequent superovulatory response. There was an increase of estradiol at 48h post PG treatment in HRG followed by decrease. The marked increase of estradiol in LRG continued till the last sample. There was a tight negative correlation between estradiol and recovered embryo in flushing day in LRG. It was concluded that higher progesterone concentration on the day of superovulatory treatment resulted in an increase in ovulation rate and embryo recovery. More over the higher estradiol level in superovulated buffalo resulted low embryo recovery rate.

Key word: Buffalo · Superovulation · PMSG · Estrogen · Progesterone

INTRODUCTION

Embryo transfer technique in buffalos was derived from those in cattle. However, the success rate is much lower in buffalos, due to their inherent lower fertility and poor superovulatory response [1,2]. Variability in the number of ovulation and the number and quality of recovered embryo produced in response to superovulation is a limiting factor in the successful application of embryo transfer in buffalo [3]. The stage of the cycle at which superovulatory treatment is initiated, the biological activity of superovulatory gonadotrophin preparation and the presence or absence of dominant follicle may all contribute to the variability in superovulatory response [4-6]. The endogenous steroid hormone may also contribute to variability in the superovulatory response in buffalo [7-9]. High estrogen level in superovulated buffalo affect the development of early embryo [8]. Also, higher progesterone concentration at the start of gonadotrophin treatment has positive effect on the subsequent ovulatory response in buffalo [10]. There are little known researches about the endocrine

Corresponding Author: Bassiouni Heleil, Department of Theriogenology,
Faculty of Veterinary Medicine, Kafr-Elsheikh University, Egypt

factors that affect the ovarian response and embryo production in superovulated buffalo. Therefore, the objective of present study was to find out the relationship between serum steroid hormonal profile and the superovulatory response in the term of number of corpora lutea and embryo recovery in Egyptian buffaloes.

MATERIAL AND METHODS

Animals: The study was carried out on buffalo herd from Mehalt Mousa farm belonging to the Animal production Research Institute, at Kafr El-Sheikh province, Egypt during the period from January to April, 2009. A total number of twenty two buffalo cows weighing 450-550 Kg and of 6-8 years age was used in this study. Animals were apparently healthy and with good body conditions. They were fed on hay, silage, concentrate mixture, barseem and rice straw. Animals were observed for at least two regular cycles before entering in the experimental program.

Ultrasonography: Transrectal ultrasonography was carried out using ultrasound scanner supplied with a 5 MHz linear array transducer (Ultra Scan 900 alliance, Quebec, Canada). All animals were examined by ultrasonography before the beginning of the experiment to exclude pregnancy and any abnormalities. Ovarian status was monitored daily through ultrasound to investigate and demonstrate the response to superovulatory treatment [11].

Superovulation: The total 22 buffaloes selected for the experiment were randomly assigned to two groups, group 1 (Ten buffaloes, as the control group) and group2 (Twelve buffaloes, as the treated group). Estrus was synchronized by I.M injection of two doses each of two ml estrumate (500ug cloprostenol, Schering-plough Animal Health Co, Germany), at an interval of 11 days. After luteolytic hormone injection, animals were carefully observed visually twice daily by well trained herd man for estrous signs. The ultrasonographic examination of the ovaries also aid in estrous detection. Pregnant mare serum gonadotrophin (Folligon, Intervet Co, B.V.Holand) was used I.M for inducing superovulation in group 2 with a dose of 3000 I U [12] on day 10 of estrous cycle. Luteolysis was induced by administration of 2ml estrumate I.M at 48 h after initiating gonadotrophin treatment. All animals were inseminated artificially two times with frozen semen at 12h intervals following detection of standing heat.

Embryo Recovery: Embryo recovery was performed non-surgically on day 5-6 [13]. It was conducted by using sterile two way Foley catheter (size 8-22). The flushing media was phosphate buffer saline (PBS) containing 1%.Bovine serum albumin (BSA). Efficacy of superovulation was determined by estimating number of corpora lutea by ultrasonography.

In the control group, all 10 animals were injected with normal saline instead of PMSG on day 10 of synchronized estrus and were inseminated as per schedule described for the experimental group.

Blood Sampling Schedule: Blood samples were collected using jugular venipuncture from all animals of two groups for determination of serum estradiol-17$_{\text{å}}$ (E2) and progesterone (P4) on day -1, 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 (Day0=day of superovulatory treatment). Blood samples were collected in glass vials and centrifuged at 3000 rpm for 15 min. Serum was decanted into glass vials and stored at -20° C until hormonal analysis. Estradiol [14] and progesterone [15] concentration were determined by validate Radio immunoassay (RIA) using kits (Diagnostic Production, Corporate Offices 5210 pacific concourse drive. Los Anglos, CA, 9004-6900 USA).

Statistical Analyses: The obtained data were represented as mean + SEM. Analysis of variance (ANOVA) was used for comparison of mean values of the various treatments at a significant level of $P<0.05$. All data were statistically analyzed according to Snedecor and Cochran [16].

RESULTS

Table 1 reveales the number of corpora lutea and recovered embryo in control and superovulated groups. In the control group, the number of CL was 1±0, while in superovulated animals, the results classified into high response group (HRG) ≥ 3 CL (7 animals, 4.85±0.33 cl) and low response group (LRG) < 3CL (5 animals, 1.8±0.19 cl). There was significant difference ($P<0.05$) between different groups regarding the number of corpora lutea. The numbers of recovered embryos were 0.3±0.15, 1.7±0.18 and 0.8±0.19 for control, HRG and LRG, respectively. There was significant difference ($P<0.05$) between HRG and each of control and LRG; however, there was no significant difference between LRG and control one.

Serum progesterone concentrations are presented in Table 2 and Fig 1.

Table 1: Ovarian response and recovered embryo in control and superovulated groups. (Mean ±SEM)

Parameter	Control	HRG	LRG
Corpora lutea	1±0 [c]	4.85±0.33[a]	1.3±0.19[b]
Recovered embryo	0.3±0.15[b]	1.7±0.18[a]	0. 8±0.19[b]

Means within the same row having different letters are significantly different at p<0.05.

HRG = High response group

LRG = Low response group

Table 2: Progesterone concentrations (ng/ml) in control and superovulated animals (Mean ±SEM)

Days of treatment	Control (n=10)	HRG (n=7)	LRG (n=5)
-1	1.11±0.11[a]	2.01±0.12[b]	1.22± 0.05[a]
0	1.2±0.12[a]	2.3±0.13[b]	1.28±0.08[a]
1	1.31±0.12[a]	2.21±0.15[b]	1.06±0.04[a]
2	1.16±0.06[a]	2.07±0.14[b]	1.06±0.05[a]
3	0.21±0.02[a]	0.38±0.02[a]	0.42±0.02[a]
4	0.17±0.01[a]	0.24±0.02[a]	0.24±0.02[a]
5	0.28±0.02[a]	0.5±0.05[b]	0.34±0.02[a]
6	0.3±0.02[a]	0.7±0.02[b]	0.5±0.04[c]
7	0.63±0.01[a]	1.8±0.06[b]	0.74±0.04[a]
8	0.88±0.02[a]	2.7±0.14[b]	1.6±0.07[c]
9	0.99±0.05[a]	3.1±0.06[b]	2.08±0.07[c]
10	1.2±0.04[a]	4.1±0.03[b]	2.3±0.08[c]

Means within the same row having different letters are significantly different at p<0.05.

HRG = High response group, LRG = Low response group

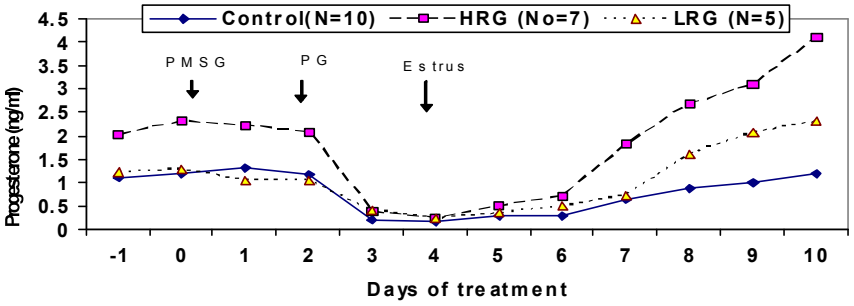

Fig. 1: Serum progesterone Concentrations in control and superovulated animals

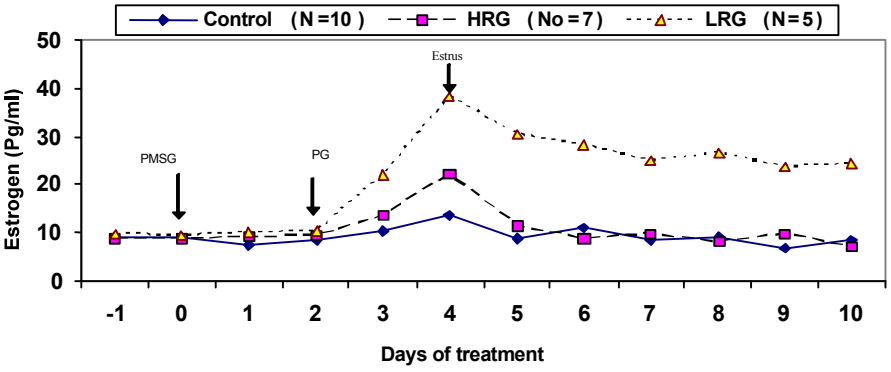

Fig. 2: Serum concentration of estradiol in control and superovulated animals

Table 3: Serum concentration of estradiol (pg/ml) in control and superovulated animals (Mean± SEM) :

Days of treatment	Control (n=10)	HRG (n=7)	LRG (n=5)
-1	9.02±0.15[a]	8.71±0.44[a]	9.8± 0.58[a]
0	9.2±0.13[a]	8.85±0.14[a]	9.4±0.51[a]
1	7.6±0.21[a]	9.1±0.26[ab]	10.2±0.37[b]
2	8.3±0.25[a]	9.57±0.37[ab]	10.4±0.67[b]
3	10.3±0.5[a]	13.71±0.49[b]	22.2±0.62[c]
4	13.7±0.44[a]	22.17±0.4[b]	38.4±3.5[c]
5	8.9±0.17[a]	11.28±0.47[b]	30.6±0.51[c]
6	11.2±0.13[a]	8.85±0.26[b]	28.1±0.75[c]
7	8.4±0.3 [a]	9.71±0.35[a]	25±0.63[b]
8	9.2±0.19[a]	8.2±0.35[a]	26.6±0.75[b]
9	6.7±0.33[a]	9.85±0.49[b]	23.8±0.66[c]
10	8.5±0.16[a]	7.2±0.28[a]	24.3±0.7[b]

Means within the same row having different letters are significantly different at p<0.05.

HRG = High response group, LRG=Low response group

Mean serum progesterone concentrations on the day of PMSG injection were significantly higher in high response group (2.3±0.13 ng/ml) than control (1.2±0.12 ng/ml) and low response group (1.28±0.08 ng/ml). The level at the initial of superovulatory treatment was significantly correlated with the number of CL (r=0.85, 0.71, P<0.05) and recovered embryos (r=0.80, 0.71, P<0.05) after superovulation in HRG and LRG, respectively. After PG2á treatment progesterone concentrations declined to less than 0.5 ng/ml within 24 hr for the three groups. From day 7 to day 10 after superovulation, there was a significant (P<0.05) rise in progesterone levels in HRG compared with that of control and LRG. Moreover, progesterone concentrations on day 10 after superovulation were positively correlated with the number of CL (r=0.81, 0.78, P<0.05) and recovered embryos (r=0.80,0.78, P<0.05) for HRG and LRG, respectively.

Serum concentrations of estradiol in control and superovulated animals were reported in Table 3 and Fig.2. The profiles did not vary significantly among control and experimental groups at the start of treatment. Also, estradiol concentrations at initiation of treatment were not correlated with the subsequent ovulation rate and recovered embryos. Peak level of estrogen (13.7±0.44 pg/ml) was obtained 48h after PG treatment in the control group. In high response group, E2 levels increased from basal concentration of 8.85±0.14pg/ml on day of PMSG injection to reach peak concentration of 22.17±0.4pg/ml on the day of superovulatory estrus. It then declined sharply till reach 7.2±0.35pg/ml on day 10 after superovulation. While, in low response group, E2 showed high level (22.2±0.62pg/ml) at 24h post PG treatment and increased sharply to the peak level of 38.4±3.5pg/ml at 48h after PG injection, thereafter the values decreased gradually but still high till the last sample with significant difference (P<0.05) with other groups. Significant negative correlation was observed between E2 and the number of CL (r=-0.87, P<0.05) and recovered embryo (r=-0.87, P<0.05) on day 10 after superovulation in low response group

DISCUSSION

The superovulatory response, expressed by number of CL in this study for high response group (HRG) and low response group (LRG) averaged4.85 ±0.33 and 1.8±0.19 CL, respectively. However, the overall mean number of CL in superovulated group was 3.58±0.49 CL. This value was nearly similar to 3.6- 4.3CL [17], but lower than 6.8CL [18] and8.8 CL [4].Overall mean number of recovered embryos in superovulated group was 1.33±0.18.This was higher than 0.05 embryos which previously reported by Ismail *et al.* [19] and lower than two embryos [8] and four embryos [20]. In this study, the very low embryo recovery rate in relation to the number of ovulation may be due to inability of fimbria to trap ova from enlarged superovulatory ovary [21], difficulties in locating hatched blastocytes [22] and premature entry of ova /embryos into the uterus, resulting into their expulsion [4]. In the same time, the very high E_2concentration in the present study may be also an important factor which lead to very low embryo recovery rate.

In the present study, the ovulation rate and embryo recovery were influenced by the progesterone concentration at the initiation of treatment. Buffalo in high response group having higher progesterone levels (2.3±0.13 ng/ml) at the initiation of gonadotrophin treatment tended to have higher ovulation rates (4.85±0.33 CL) and embryo recovery (1.71±0.18). These results are in

agreement with the previous results which reported a positive correlation of superovulatory response with circulatory progesterone at time of gonadotrophin treatment [10,23,24]. The same results were obtained by Madan *et al*. [25] who mentioned that, animals with >2ng/ml plasma p4 during pretreatment has better superovulatory treatment than those having <2 ng/ml p4.However, the present findings were contradictory with that of Ullah *et al*. [21] who fail to establish any correlation between p4 concentration on the day of FSH treatment and superovulatory response in buffaloes. Several studies have indicated that progesterone levels declined to below 0.5 ng /ml within 24h of PG treatment [8,10] and coincide with the present findings. A significant rise in progesterone levels on 72h in high response group after estrus following superovulation, compared with that of control and low response groups was probably due to the secretion of progesterone by multiple CL and also to the lutinization of a large ovulated follicle [26]. In the present study, progesterone levels on day 10 (day of flushing) after superovulatory were positively correlated with the number of CL and recovered embryoes. The same result was obtained by Misra *et al.* [10].

Serum estradiol concentration in the present study didin't vary among control and experimental groups at the start of treatment, moreover, it was not correlated with the subsequent superovulatory response. This result confirms the result of Testart *et al*. [27]. The two fold increase in estradiol 17 B concentrations observed at 24 h post PG treatment in low response group in the current study was confirmed by the finding of other workers [8,25]. Higher peak estradiol levels in superovulated than in control buffaloes is due to multiple follicular growth induced by superovulation [28]. In the present result, the very high estradiol concentration and its longer duration seen in buffaloes in low response group may be involved in poor embryo recovery rate observed in this group, these results were in agreement with the previous results which mentioned that high estrogen level in superovulated buffaloes affect the development of early embryo [8].

It was concluded that higher progesterone concentration on the day of superovulatory treatment resulted in an increase in ovulation rate and embryo recovery. On the other hand, serum estradiol levels at the initiation of gonadotrophin treatment did not affect the superovulatory response, while, higher estradiol levels in superovulated buffalo after superovulatory estrus resulted in poor embryo recovery rate.

REFERENCES

1. Techakumphu, M., Y. Sukawong and M. Intraramongkol, 2001. The effect of gonadotrophin releasing hormone on superovulation in Elite swamp Buffalo cow (Bubalus bubalis). J. Vet. Med. Sci., 63: 853-857.
2. Manik, R.S., P. Palta, S.K. Singla and M.N. sharma, 2002. Folliculogenesis in buffalo (Bubalus bubalis). J. Reprod. Fert., 14: 315-325.
3. Misra, A.K. and S. Tyagi, 2007. *In vivo* embryo production in buffalo:present and perspectives. Ital. J. Anim. Sci., 6: 74-91.80.l
4. Karaivanov, C., D. Kacheva, M. Petrov, K. Vlakhov and E. Sapundjer, 1990. Superovulation response of River buffalo. Theriogenology, 33: 453-464
5. Taneja, M., S.M. Totey, C.H. Pawshe, G. Singh, A. Ali and G.P. Talwar, 1991. Ultrasonographic monitoring of ovarian follicular dynamics in superovulated buffaloes. Proc. Ø World Buffalo Congress, Bulgaria pp: 581-585.
6. Techakumphu, M., C. Lohachit, W. Srianan, C. Intaramongkol and P. Chantaraprateep, 1996. Ovarian response and oocyte recovery in prepubertal (Bubalus bubalis) calves after FSH or PMSG treatment. Theriogenology, 45: 244-251.
7. Herrler, A. and H. Niemann, 1989. Rapid milk progesterone assay as a tool for screening potential donor cows prior to superovulation. Theriogenology, 31: 203-207.
8. Schallenberger, E., R. Wagner, P. Papa, H. Hartl and H. Tenhumerg, 1990. Endocrinology evaluation of the induction of superovulation. Theriogenology, 34: 379-392.
9. Singh, C. and M.L. Madan, 1999. The ovarian response of prepubertal buffaloes (Bubalus bubalis) to superovulation with equine chorionic gonadotrophin with and without treatment with GnRH.The Veterinary Journal, 158: 155-158.
10. Misra, A.K., R.S. Jaiswal, R. Kasiraj, M. MuthaRao, N.S. Rangareddy and H.C. Pant, 2000. Periovulatory steroid hormone profiles during unstimulated and superovulatory oestrus cycle in buffalo (Bubalus bubalis). Buffalo J., 3: 371-3
11. Baruselli, P.S., R.G. Mucciolo, R. Amaral, R. Arruda, M.E.O.A. Assumpcao, E.H. Madureira and J.A. Visintin, 1998. Taxa de recuperacao embrionaria em bufalas superovuladas In: Runiao annual da sociedade brasileira De Transferencia De Embrioes, 13, Atibaia,. Arq. Fac. Vet. UFRGS, V. 26, 22 (abstr.)

12. Heleil B., El-S.M Fattouh, B.H. Serur, S.A. Darwish and Y.M. El-Deeb, 2009. Superovulation withPMSG (eCG) and embryo Transfer of Egyptian buffalo. Kafrelsheikh Vet. Med. J., 7: 174-199
13. Newcomb, R., W.R. Christie and L.A. Rowson, 1978. Non surgical recovery of bovine embryo. Vet., Rec., 13: 414-117.
14. Singh, C. and M.L. Madan, 1998. Pituitary and gonadal response to GnRH in prepubertal buffaloes (Bubalus bubalis). Asian-Austral. J. Anim. Sci., 11: 78-83.
15. Prakash, B.S. and M.L. Madan, 1986. Peripheral plasma estradiol 17B progesterone and cortisol in buffaloes induced to calve dexamethason and vetoestrol. Anim. Reprod. Sci., 11: 111-22.
16. Snedecor, G.W. and W.G. Cochran, 1980. Statistical methods. 7th Ed., The Iowa State Univ., Press. Amer. Iowa, USA.
17. Drost, M., K. Vlakhov, W.S. Cripe and M. Petrov, 1988. Successful non surgical embryo transfer in buffalo in Bulgaria. Theriogenology, 30(4) : 659-668.
18. Situmorang, P., 2005. Effect the administration of human chrionic gonadotrophin (hCG) hormone following superovulation treatment in buffalo. JITV, 10: 286-292.
19. Ismail, S.T., M.Y. Abboud, M.S. Tawfik, K.M. Mohamed and H. Shawky, 1992. Effect of PMSG and FSH on the ovarian response and embryo production in buffaloes. Proc. 4th Annual Egyptian Soc. Anim. Reprod. Fert. Cong., Cairo, Egypt, pp: 39-49.
20. Hesheng, J., Y. Wei, Q. Jiang, J. Wei, Z. Wei, P. Wei, B. Xei, H. Huang and X. Hao, 2006. Pregnancies resulted from transfer of in-vivo embryos derived from nonsurgical recovery in superovulated buffaloes.Proc.5th Asian Buffalo Congress, Nanning.China, pp: 53-58.
21. Ullah, N., R.W. Wright, A. Mehmood and S.M. Baig, 1992. Endocrine profile in relation to ovarian response, recovery rate and quality of embryos in Nili-Ravi buffaloes treated with FSH. Buffalo J., 1: 47-56.
22. Alexiev, A., K. Vlakhov, C.H. Karaivanov, D. Kacheva, M. Petrov, N. Nikolov, A. Drogoev and P. Radev, 1988. Embryo transfer in buffaloes in Bulgaria. II World Buffalo Congress, New Delhi, II: 591-595.
23. Tuyen, D.K., S. Jailkhani, M.L. Madan, B.S. Prakash and S.K. Siongla, 1990. Progesterone and estrogen profile during superovulation in buffaloes. Theriogenology, 33: 340.
24. Prakash, B.S., S.K. Singla, J.D. Ambrose, S. Jailkhani and M.L. Madan, 1992. Assessment of superovulatory resposees in terms of palpable corpora lutea and embryo recovery using milk progesterone. Theriogenology, 37: 897-905.
25. Madan, M.L., S.K. Singla, C. Singh, B.S. Prakash and S. Jailkhani, 1988. Embryo transfer technology in buffaloes:Endocrine response and limitations. Proc.Ïnd World Buff. Cong. Physiol and Reprod., 2: 195-211.
26. Ireland, I.J. and J.F. Roche, 1982. Development of antral follicles in cattle after prostaglandin induced luteolysis:Changes in serum hormones, steroids in follicular fluid and gonadotrophin receptors. Endocrinology, 111: 2077-2086.
27. Testart, J., G. Kann, J. Saumande and M. Mubier, 1977. Estradio 17, progesterone, FSH and LH in prepupertal calves induced to superovulate. J. Reprod. Fert., 51: 329-336.
28. Bittridge, K.J., 1977. Embryo transfer in farm animals. Areview of techniques and applications.Canada Department of Agriculture Monograph, 16: 1-34.