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Effects of Pre-Treatment with GnRH on the Efficiency of Superstimulatory Protocol in Water Buffalo (*Bubalus bubalis*)

Research Article

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ABSTRACT

The multiple ovulation embryo transfer technique (MOET) in buffalo has yielded poor superovulation response due to presence of large/dominant follicle(s) at the start of superstimulatory treatment. The aim of present study was to evaluate the effects of pretreatment with gonadotropin on number of total as well as viable and transferrable embryos recovered during MOET programme in Murrah buffaloes. Buffaloes (n=27) enrolled into two treatment (groups A and B, n=9 in each group) and one control (group C, n=9) groups were administered with 600 mg of follicle stimulating hormone (FSH) in 10 decreasing doses at 12 hourly interval for five days. Additionally, buffaloes were administered with a single dose of GnRH in group A (10 µg) and group B (06 µg) as a pre-treatment on 2.5 day prior to the start of FSH. Two doses of prostaglandin (500 µg) were administered with the 7th and 8th dose of FSH in all the buffaloes. Embryos were collected non-surgically on day 5.5 post-insemination. Number of total, transferrable and the non-transferrable embryos recovered were recorded in the 3 groups. Total as well as the viable transferrable number of embryos recovered were significantly higher ($P<0.05$) in the buffaloes of group B compared to the control (3.0 *vs.* 1.33 and 2.33 *vs.* 1.0, respectively). In all the three groups, about 75% of embryos recovered were of transferrable grade. The results of study indicated that administration of GnRH as a pre-treatment in superstimulatory treatment improved the MOET efficiency in buffalo.

KEY WORDS buffalo, embryo, follicle stimulating hormone, GnRH.

INTRODUCTION

Livestock sector is an integral component of agriculture farming system and have a pivotal role in the rural economy of India. The water buffalo is a premier dairy animal in India and many other south Asian countries. Poor reproductive performance of buffaloes due to delayed puberty, silent estrus, poor conception rates, long postpartum interval etc. causes heavy economic losses to the livestock farmers. This led to diminishing interest of the farmers in buffalo husbandry as indicated by declining growth rate of buffalo population during the last decade. Amongst the various reproductive biotechniques multiple ovulation embryo transfer technology (MOET) could be a viable option to augment the need of faster genetic gain for making buffalo farming profitable. The suitable superstimulatory and embryo flushing protocols for buffaloes are ideally different from cattle due to differences in species, ovarian follicular population and dynamics; and developmental rate and transport of early embryo. Generally, a greater dose of FSH

(600 mg) in decreasing schedule for buffaloes for superstimulation and the embryo flushing on day 5.5 after artificial insemination (AI) was recommended (Misra *et al.* 1998). Furthermore, the number of ovulations, total and viable transferrable embryos recovered were lesser in buffalo compared to cattle (Baruselli *et al.* 2018). The aforesaid parameters are largely affected by the recruitment of follicular wave, presence of dominant follicle and progesterone milieu present at the start of superstimulatory treatment (Misra and Tyagi, 2007). Presence of dominant follicle at initiation of ovarian super-stimulation have a negative (Kohram and Poorhamdollah, 2012), whereas higher progesterone concentration have a positive effect (Ravi *et al.* 2011) on the outcomes of superstimulation. During the last decades, a few approaches were proposed to manipulate the ovarian follicular dynamics to circumvent the effect of dominant follicle so as to improve the success of MOET. Ablation of the dominant follicle (Leonardo *et al.* 2019), manipulation of the estrus cycle (by progesterone and oestradiol preparations; (Bulbul *et al.* 2010; Bulbul *et al.* 2013) and pre-treatment with gonadotropin releasing hormone (GnRH) (Sato *et al.* 2005) have been successfully used to manipulate and induce the emergence of a new follicular wave to optimize the superovulatory outcomes in cattle. Administration of GnRH causes ovulation/luteinization of the dominant follicle (Pursley *et al.* 1995) and 2-3 days later emergence of a new follicular wave (Naseer *et al.* 2012). The applicability of this aspect has not been evaluated during superstimulation in buffaloes. Moreover, ovary on the 7 or 8 day after end of estrus would probably have a large dominant follicle responsive to exogenous gonadotropin (Baruselli *et al.* 2013), irrespective of buffalo having whether 2-wave or 3-wave follicular dynamics. We hypothesised that GnRH treatment on 7.5 day post-estrus would alleviate the negative effect of dominant follicle and improve the superstimulatory attributes when we start superovulatory treatment 2.5 day later to it. Accordingly, the present study was conducted to evaluate the effect of pre-treatment with two different doses of GnRH on the total and viable embryo recovery in buffalo.

MATERIALS AND METHODS

Animal selection

Twenty seven Murrah buffaloes (*Bubalus bubalis*) maintained at the institutional dairy farm were assigned into this study. Buffalo enrolled into the study were in the second to fourth parity, lactating, had a mean age of 6.2 ± 1.4 years, weighing 450-560 kg, minimum 70 days post-partum and a body condition score (BCS) of 3.5–4.0 (5-point scale; Alapati *et al.* 2010). Healthy normal cyclic buffaloes with healthy genitalia and patent cervix during diestrus were selected based on the gynaecological and trans-rectal ultrasonographic examinations. Animals were provided with *ad libitum* drinking water and daily rations comprising of fresh green fodder, straws and concentrates. The experimental protocol was approved by the institutional animal ethics committee.

Experimental design

Buffaloes were randomly selected for two treatment groups (group A and B) and one control group (group C). Each treatment group (n=9) was subjected to a different dose of GnRH as a pre-treatment strategy. Buffaloes were observed for spontaneous estrus or those with palpable mature corpus luteum were induced to estrus with prostaglandins ($PGF_{2\alpha}$; 500 µg; cloprostenol sodium, intramuscularly). The estrus was detected at 12 h interval following 48 h after $PGF_{2\alpha}$ injection by close observation of behavioral external signs (bellowing, mounting, frequent micturition, cervico-vaginal mucus discharge, swollen and edematous vulva) and was confirmed by per-rectal examination of uterine tonicity, cervico-vaginal discharge and its arborization. A schematic schedule of protocols used for *in vivo* embryo production is depicted in Figure 1. On seventh day after the end of estrus, pretreatment with gonadotropin (GnRH) was administered intramuscularly in two groups with different doses. i.e. 10 µg GnRH in group A (n=9) and 06 µg GnRH in Group B (n=9), while group C (control group) received an injection 2 mL of distilled water as a placebo. Superstimulatory treatment was started in buffaloes of all the three groups on day 10 after the end of estrus. All buffaloes were injected with 600 mg follicle stimulating hormone intramuscularly (FSH; Folltropin-V®, Bioniche Animal Health Inc., Canada) in 10 divided decreasing doses (80:80, 70:70, 60:60, 50:50, 40:40 mg) at 12 hr interval (morning and evening) over the next five day period. Prostaglandin (cloprostenol sodium, 500 µg intramuscularly) was given with seventh and eighth dose of FSH for luteolysis in order to induce estrus 36-48 h later. Fixed timed artificial inseminations (AI) were done thrice at 12 h interval starting from 36 h of the first prostaglandin injection using good quality frozen-thawed semen (with at least 10 million motile spermatozoa) of an elite Murrah buffalo bull.

Embryo flushing and evaluation

Superovulatory response in terms of number of corpus lutea (CL) formed was assessed manually prior to embryo flushing. Flushing of embryos from the uterus on 5.5 day post-first AI was done by non-surgical method under mild sedation (xylazine hydrochloride 5-10 mg) and epidural anesthesia (lignocaine hydrochloride 40-100 mg) using 18 G silicon catheter as described previously (Singhal, 2010). In brief, each uterine horn was flushed using about 500 ml of flushing medium (Dulbecco's phosphate buffer saline) containing 0.1% bovine serum albumin.

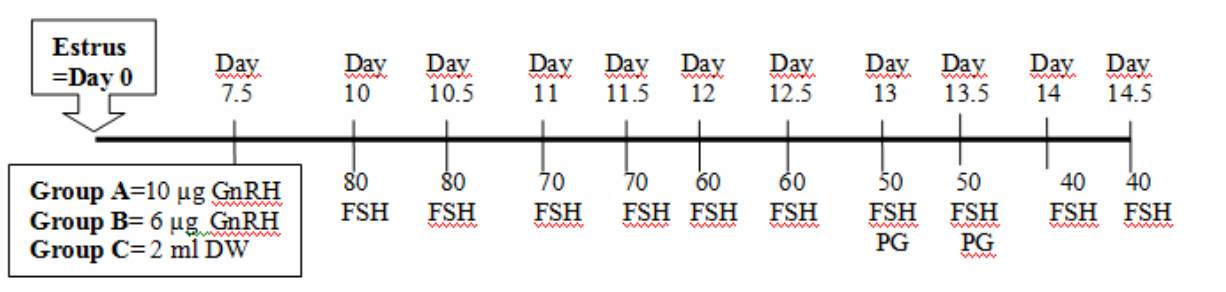

Figure 1 Time line for the experimental groups in superstimulatory protocol
FSH: follicle stimulating hormone (in mg) and PG: prostaglandin and DW: distilled water

After completing the process of embryo flushing, antibiotic suspension (30 mL of 4% gentamicin sulphate solution) was infused into the uterus as a precautionary measure to protect against uterine infection. A luteolytic agent was administered intramuscularly to bring the animal in estrus again. Flushed out media was collected in an Emcon filter and transferred into petridish (90×10 mm; Nunc, Thermo Fisher Scientific, USA).

A thorough searching for embryos was undertaken using stereozoom microscope (10-50×magnification) and the embryos were categorized as of viable transferrable and non-transferrable quality according to standard classification (Phillips and Jahnke, 2016).

Statistical analyses

Statistical software (SPSS, 2011) was used for the making the statistical comparisons of the data. Difference between means was compared using students 't' test. The percent of transferrable embryos between the groups were compared using Chi-square test. $P < 0.05$ were considered to be statistically significant. The findings obtained in this study are presented as mean values and percentages. All mean values are expressed as the mean ± standard error of mean (Table 1).

RESULTS AND DISCUSSION

The present study evaluated the effects of additional pretreatment with GnRH on the outcomes of superstimulation during MOET in buffaloes. Two treatment groups i.e. groups A and B were treated with 10 and 06 µg GnRH, respectively whereas group C was administered with 2 mL DW to serve as control. Variables pertaining to superovulatory response viz. (mean number of corpora lutea, mean number of total embryos recovered, mean number of transferrable embryos, mean number of non-transferrable embryos, percentage of transferrable embryos) obtained in all the groups are depicted in Table 1.

Superovulatory response

Superovulatory response (SOR) was evaluated by counting the total number of corpus lutem (CL) palpable per-rectally over both the ovaries. Both the experimental groups treated with GnRH showed higher SOR than control and group B (GnRH pretreatment @ 06 µg) differs significantly ($P<0.05$) with control (Table 1).

Total embryos recovery

The embryo recovery rate was calculated as = total number of ova and embryos recovered/number of CL detected × 100. A total of 27 uterine flushings (nine in each group) for embryo collection were executed in the study and a total of 58 embryos were recovered. The average total number of embryos recovered per group were 2.11 ± 0.61, 3.0 ± 0.71 and 1.33 ± 0.33, respectively in group A, B and C. GnRH pretreatment (06 µg IM) showed significantly higher ($P<0.05$) total embryo recovery in comparison to control group. In the similar pattern, buffaloes of group B have highest transferrable embryos per flushing (2.33±0.64) which was significantly higher ($P<0.05$) than control but didn't differ significantly from other GnRH pretreated group A. Pooled data of all groups revealed that the mean total and average viable transferrable embryos were 2.15 ± 0.34 and 1.63 ± 0.30, respectively per animal.

Recovery of viable transferrable embryos

In the study, percentage of transferrable embryos recovered from total embryos was 73.7, 77.8 and 75% for group A, B and C, respectively. Percent transferrable recovery and non-transferrable embryos per flushing did not differ significantly ($P<0.05$) among the three experimental groups.

Recovery of poor quality embryos

The current study observed non-transferrable or poor quality embryos were 0.55 ± 0.18, 0.66 ± 0.23 and 0.33 ± 0.17 in group A, B and C, respectively. These values were statistically similar in the three experimental groups.

Table 1 Superstimulatory response in buffaloes pretreated with different doses of GnRH (Mean±SEM)

Parameters	Groups		
	Group A (GnRH, 10 µg)	Group B (GnRH, 06 µg)	Group C (control)
Mean number of corpora lutea per donor	7.0±0.65^{ab}	8.4±0.72^{b}	6.17±0.45^{a}
Mean number of total embryo(s) recovered per flushing	2.11±0.61^{ab}	3.0±0.71^{b}	1.33±0.33^{a}
Mean number of transferrable embryo(s) per flushing	1.55±0.53^{ab}	2.33±0.64^{b}	1.0±0.29^{a}
Mean number of non-transferrable embryo(s) per flushing	0.55±0.18	0.66±0.23	0.33±0.17
Percent of transferrable embryo(s)	73.68%	77.78%	75.0%

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Recovery of poor quality embryo must be low for improved efficiency of MOET as indicated in this study compared to other reports, which may be caused by various genetic or non-genetic factors involved in different studies (Misra and Tyagi, 2007).

In this study, we demonstrated the effects of pretreatment by GnRH on superstimulatory variables in water buffaloes. In the current study, the average recovery of 2.15 embryos per flushing (58/27) was recorded and is higher than results of other study during presence of dominant follicle but is equivalent if superstimulation was done using PMSG (Folligon, 3000 IU) in absence of dominant follicle (Heleil and El-Deeb, 2010). The total number of embryos recovered per animal in present study is in agreement with the equivalent recovery of average 1-3 embryo reported earlier using 400 mg Folltropin (Redhead *et al.* 2018), but lower than about four embryos obtained per animal in another study (Hesheng *et al.* 2006). Neglia *et al.* (2010) showed similar total recovery of 2.3-2.8 embryos per buffalo using PRID and 500 IU of FSH while very poor recovery has been reported in heifers (Kandil *et al.* 2012). These discrepancies in embryo recovery might due to differences in weather conditions (Phogat *et al.* 2016), age, parity and lactational status of donor (Misra and Tyagi, 2007).

The present study observed significantly (P<0.05) greater mean number of corpora lutea in treatment group B compared to control (8.4±0.72 *vs*. 6.17±0.45). On same pattern, mean total embryo recovery in group B was significantly (P<0.05) higher (3.0±0.71) than mean embryo recovered (1.33±0.33) from control experimental group (Table 1). In current study, embryo recovery rate in relation to number of ovulation(s) is lesser which could be attributed to the inability of fimbria to trap ova from enlarged superovulatory ovary, difficulty in locating hatched blastocytes and or premature entry of ova/embryos into the uterus (Karaivanov *et al.* 1990; Ullah *et al.* 1992). The altered biochemical milieu during the superovulation might also result in impaired ovulation (Angela *et al.* 2018) and the oviductal transport (Carvalho *et al.* 2006).

Although much higher than many earlier reports, the percentage of viable transferrable grade embryos yielded (about 75%) in the present study is similar in all the 3 groups. Similarly, 50-80% of embryos recovered were of transferrable quality with or without GnRH treatment during estrus (Techakumphu *et al.* 2001). The varied viable embryo recovery rate from 32.6% to 84.4% in superovulated buffaloes inseminated using the frozen-thawed semen of five different bulls have also been reported (Misra *et al.* 1999). Variation in agro-climatic zones or the use of semen of a single elite bull might be the cause of reasonably greater proportion of transferrable grade embryos obtained in the present study. However, the overall yield of average viable and transferable embryos (1.63; 44/27) per buffalo from the 27 flushings recorded in the present study is very similar to earlier observation (1.62; Kandil et *al.* 2012). These results are in concurrence with several other reports of an average yield of 1-3 viable embryos per flushing (Baruselli *et al.* 2013). In all the three groups, we noticed that the quality of embryos was better when embryos were recovered in multiple number (>1) rather than the recovery of single embryo in a uterine flushing. This effect may be due to cell to cell communication signals which promote mutual growth and development of the developing embryos, leading to improved quality in the cohort. Correspondingly, better *in vitro* developmental rates of oocytes and early embryos have been observed when cultured in groups compared to the individual (Salvador *et al.* 2011).

GnRH has important role in regulation of the recruitment, selection, dominance and ovulation of follicles. These events have been induced with exogenous gonadotropins in gonadotropin deprived sheep (Driancourt *et al.* 1987). There is limited information available in the literature regarding utilization of GnRH pretreatment prior to the superstimulatory protocol in buffalo. Use of the GnRH obviate the effect of dominant follicle either by causing its ovulation or leutinization during estrus synchronization in bovine (Mohammadi *et al.* 2019) and during MOET in cattle (Sato *et al.* 2005).

Therefore, GnRH would increase the progesterone concentration by increasing luteal tissue and may also recruit a new follicular wave within two to three days. It was indicated that administration of GnRH induced the emergence of a new follicular wave within 2-3 days after the induced ovulation of dominant follicle in cattle (Macmillan and Thatcher, 1991) and in about 60% of buffalo (Baruselli *et al*. 2013). Present study observed, the mean CL number were greater in both the experimental groups compared to the control group, however, it didn't vary significantly among GnRH treated groups and was numerically higher with lower dose treatment. In the current study, FSH treatment given on 2.5 days after the GnRH might have coincided with a newly recruited follicular wave and lead to better superovulatory outcomes both in terms of embryo recovery rate as observed in the treatment groups compared to control group. In accordance, a lesser dose of GnRH given 2.5 days prior to superstimulation showed improved superovulatory outcomes in cattle (Sato *et al*. 2005). Similarly, increased ovulation rate and embryo recovery were observed in absence of the dominant follicle in cattle (Bungartz and Niemann, 1993) and buffalo (Honparkhe *et al*. 2014). Contrarily, there was no difference in superovulatory response in cows when the superstimulatory treatment was initiated in presence or absence of dominant follicle (Wilson *et al*. 1990). Moreover, endogenous release of LH is dependent on dose of GnRH administered exogenously (Veldhuis *et al*. 1989). In the present study, lower dose of GnRH (06 µg) improved the superovulatory outcomes because buffaloes are more sensitive to exogenous hormonal therapy than cattle (Nanda *et al*. 2003). This finding is supported by the observation that a higher dose of GnRH agonist reduced sensitivity and number of receptors of GnRH on hypothalamus (Ulker *et al*. 2001), which in turn suppresses the endogenous secretion of gonadotropins.

CONCLUSION

In conclusion, the study indicated that the application of pretreatment with GnRH on 2.5 days prior to start of the superstimulatory protocol improved the yield of total and viable grade embryos. Hence, further elaborate experiments are warranted to establish the beneficial effect of GnRH pretreatment to replace the conventional superovulatory protocols for improving efficiency of MOET in buffaloes.

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REFERENCES

Alapati A., Kapa S.R., Jeepalyam S., Rangappa S.M. and Yemireddy K.R. (2010). Development of the body condition score system in Murrah buffaloes: Validation through ultrasonic assessment of body fat reserves. *J. Vet. Sci*. **11,** 1-8.

Angela S., Carolina D.C., Floriana D.R., Alberto P., Gianluigi Z., Gianluca N. and Bianca G. (2018). Evaluation of factors involved in the failure of ovum capture in superovulated buffaloes. *Theriogenology*. **122,** 102-108.

Baruselli P.S., Soares J.G., Bayeux B.M., Silva J.C.B., Mingoti R.D. and Carvalho N.A.T. (2018). Assisted reproduction technologies (ART) in water buffaloes. *Anim. Reprod*. **15(1),** 971-983.

Baruselli P.S., Soares J.G., Gimenes L.U., Monteiro B.M., Olazarri M.J. and Carvalho N.A.T. (2013). Control of buffalo follicular dynamics for artificial insemination, superovulation and *in vitro* embryo production. *Buffalo Bull.* **32,** 160-176.

Bulbul B., Kirbas M., Dursun S. and Kose M. (2013). Superovulation in cows synchronized with two different progesterone+oestradiol protocols. *Arch. Tierz.* **55,** 161-169.

Bulbul B., Kirbas M., Kose M., Dursun S. and Colak M. (2010). Investigation of superovulation response in cows by manipulation of the oestrus cycle using progesterone and oestradiol, *Kafkas Üniv. Vet. Fak. Derg.* **16(3),** 463-468.

Bungartz L. and Niemann H. (1993). Effects of a dominant follicle on ovarian responses of dairy cows following various superovulatory treatment schedules. *Theriogenology.* **39,** 198-204.

Carvalho N.A.T., Nichi M., Gallupo A.G., Melo G.M., Souza R.J., DAngelo M. and Baruselli P.S. (2006). Oviduct ciliary currents in bovine and buffalo at day 1after ovulation. *J. Reprod. Fertil. Dev.* **18,** 287-288.

Driancourt M.A., Fry R.C., Clarke I.J. and Cahill L.P. (1987). Follicular growth and regression during the 8 days after hypophysectomy in sheep. *J. Reprod. Fertil.* **79,** 635-644.

Heleil B. and El-Deeb Y. (2010). Superovulatory response in relation to follicular dynamics and presence of dominant follicles in Egyptian buffaloes. *Adv. Biol. Res.* **4,** 169-174.

Hesheng J., Yingming W., Quinyang J., Jingwei W., Zhijian W., Peijun W., Bingkun X., Haipeng H. and Xiao H. (2006). Pregnancies resulted from transfer of *in vivo* embryos derived from nonsurgical recovery in superovulated buffaloes. Pp. 53-58 in Proc. 5th Asian Buffalo Congr., Nanning, China.

Honparkhe M., Gandotra V.K., Matharoo J.S., Ghuman S.P.S., Dadarwal D. and Singh J. (2014). Synchronization of follicular wave emergence following ultrasound guided transvaginal follicle ablation or estradiol-17β administration in water buffalo (*Bubalus bubalis*). *Anim. Reprod. Sci.* **146(1),** 5-14.

Kandil O.M., Abdoon A.S.S. and Kacheva D. (2012). Successful embryo transfer in Egyptian buffaloes. *Glob. Vet.* **8,** 320-327.

Karaivanov C., Kacheva D., Petrov M., Vlahov K. and Sapundjiev E. (1990). Superovulatory response of river buffalo (*Bubalus bubalis*). *Theriogenology.* **33,** 453-464.

Kohram H. and Poorhamdollah M. (2012). Relationships between the ovarian status and superovulatory responses in dairy cattle.

Anim. Reprod. Sci. **131,** 123-128.

Leonardo P.S., Diego D.M., Aldemar C.R., Juan G.J. and Diego V.A. (2019). Superovulatory response in Brahman donor cows using follicular ablation before superovulation protocols. *Rev. MVZ Cordoba*. **24,** 7203-7208.

Macmillan K.L. and Thatcher W.W. (1991). Effects of an agonist of gonadotropin-releasing hormone on ovarian follicles in cattle. *Biol. Reprod.* **45,** 883-889.

Misra A.K. and Tyagi S. (2007). *In vivo* embryo production in buffalo: current situation and future perspectives. *Italian J. Anim. Sci.* **6,** 74-91.

Misra A.K., Kasiraj R., Mutha Rao M., Rangareddy N.S., Jaiswal R.S. and Pant H.C. (1998). Rate of transport and development of preimplantation embryo in the superovulated buffalo (*Bubalus bubalis*). *Theriogenology.* **50,** 637-649.

Misra A.K., Mutha R.M., Kasiraj R., Rangareddy N.S. and Pant H.C. (1999). Bull specific effect on fertilization rate and viable embryo recovery in the superovulated buffalo (*Bubalus bubalis*). *Theriogenology.* **52,** 701-707.

Mohammadi A., Seifi H.A. and Farzaneh N. (2019). Effect of prostaglandin $F_{2\alpha}$ and GnRH administration at the time of artificial insemination on reproductive performance of dairy cows. *Vet. Res. Forum.* **10,** 153-158.

Nanda A.S., Brar P.S. and Prabhakar S. (2003). Enhancing reproductive performance in dairy buffalo, major constraints and achievements. *Reproduction.* **61,** 27-36.

Naseer Z., Ahmad N., Khan M.I.R., Ahmad E., Tahir M.Z. and Singh J. (2012). Effect of GnRH and estradiol benzoate on follicular wave emergence, estrus, ovulation and pregnancy rate in CIDR treated Nilli Ravi buffaloes. *J. Anim. Plant Sci*. **22,** 142-146.

Neglia G., Gasparrini B., Vecchio D., Di Palo R., Zicarelli L. and Campanile G. (2010). Progesterone supplementation during multiple ovulation treatment in buffalo species (*Bubalus bubalis*). *Trop. Anim. Health Prod.* **42,** 1243-1247.

Phillips P.E. and Jahnke M.M. (2016). Embryo transfer (techniques, donors and recipients). *Vet. Clin. Food Anim.* **32,** 365-385.

Phogat J.B., Pandey A.K. and Singh I. (2016). Seasonality in buffalo reproduction. *Int. J. Plant Anim. Environ. Sci.* **6,** 46-55.

Pursley J.R., Mee M.O. and Wiltbank M.C. (1995). Synchronization of ovulation in dairy cows using $PGF_{2\alpha}$ and GnRH. *Theriogenology.* **44,** 915-923.

Ravi S.K., Prasad S., Singh B., Prasad J.K. and Singhal S. (2011). Hormonal profile in superovulated buffaloes following ablation of dominant follicle. *Indian Vet. J.* **88,** 25-27.

Redhead A.K., Siewb N., Lambie N., Carnarvon D., Ramgattie R. and Knights M. (2018). The relationship between circulating concentration of AMH and LH content in the follicle stimulating hormone (FSH) preparations on follicular growth and ovulatory response to superovulation in water buffaloes. *Anim. Reprod. Sci.* **188,** 66-73.

Salvador I., Cebrian-Serrano A., Salamone D. and Silvestre M.A. (2011). Effect of number of oocytes and embryos on *in vitro* oocyte maturation, fertilization and embryo development in bovine. *Spanish J. Agric. Res.* **9,** 744-752.

Sato T., Nakada K., Uchiyama Y., Kimura Y., Fujiwara N., Sato Y., Umeda M. and Furukawa T. (2005). The effect of pretreatment with different doses of GnRH to synchronize follicular wave on superstimulation of follicular growth in dairy cattle. *J. Reprod. Dev*. **51,** 512-515.

Singhal S. (2010). Hormonal manipulation of dominant follicle and its effect on superovulation in buffaloes. Ph D. Thesis. G.B. Pant University of Agriculture and Technology, Pantnagar, India.

SPSS Inc. (2011). Statistical Package for Social Sciences Study. SPSS for Windows, Version 20. Chicago SPSS Inc., USA.

Techakumphu M., Sukavong Y., Intaramongkol S. and Intaramongkol J. (2001). The effect of gonadotropin releasing hormone on superovulation in elite swamp buffalo cows (*Bubalus bubalis*). *J. Vet. Med. Sci.* **63,** 853-857.

Ulker H., Kanter M., Gokdal O., de Avila D.M. and Reeves J.J. (2001). The effects of recombinant LHRH fusion proteins on testicular development and histology in ram lambs. *Deutsche Dtsch. Tierärztl. Wochenschr.* **108,** 459-464.

Ullah N., Wright Jr R.W., Mehmood A. and Baig S.M. (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.

Veldhuis J.D., O'Dea L.S. and Johnson M.L. (1989). The nature of the gonadotropin-releasing hormone stimulus-luteinizing hormone secretary response of human gonadotrophs *in vivo*. *J. Clin. Endocrinol. Metab*. **68,** 661-670.

Wilson J.M., Jones A.L. and Miller D.R. (1990). Influence of a dominant follicle on the superovulatory response. *Theriogenology.* **33,** 349-357.