Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
Accessible at: www.pvj.com.pk

RESEARCH ARTICLE

Estrus Synchronization and Conception Rates Using Locally Prepared Methylacetoxy-progesterone Sponges in Cyclic and Acyclic Nili-Ravi Buffaloes (*Bubalus bubalis*)

R Kausar*, SA Khanum, M Hussain, T Hussain, N Ahmad[1], L Ahmad[2] and NA Qureshi[2]

Animal Sciences Division, Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad;
[1]Department of Theriogenology, University of Agriculture, Faisalabad; [2]Department of Wildlife and Fisheries, Government College University, Faisalabad, Pakistan
*Corresponding author: rehana_niab@yahoo.com

ARTICLE HISTORY

Received: February 9, 2013
Revised: June 28, 2013
Accepted: July 13, 2013

Key words:
Buffalo
Conception rate
Estrous cycle
Estrus synchronization
Methylacetoxyprogesterone sponges

ABSTRACT

A study was conducted to evaluate the efficacy of locally prepared intra-vaginal methylacetoxyprogesterone (MAP) sponges for estrus synchronization and conception rate in cyclic and acyclic Nili-Ravi buffaloes. In the first experiment, 20 buffaloes were divided into treated and control groups (n: 10 each) and again sub-grouped into cyclic (n: 4) and acyclic (n: 6) lots in each group, depending on their ovarian status. MAP sponges were placed in the vagina of buffaloes of treated groups for 16 days. After sponge removal, buffaloes were monitored for estrus. Serum progesterone concentrations were determined in synchronized animals for one estrous cycle using radioimmunoassay. Overall, synchronization rate in MAP-treated groups was significantly higher ($P<0.05$) as compared to controls (60 vs. 0%). However, it differed non-significantly between cyclic and acyclic buffaloes of treated groups (75 vs. 50%). At estrus, mean serum progesterone in synchronized animals was at basal level (0.26±0.08 and 0.32±0.27 $ngml^{-1}$ in cyclic and acyclic treated groups, respectively). It attained peak levels of 3.00±1.80 to 3.45±0.44 $ngml^{-1}$ on d-14, then dropped on d-18 and declined to basal values at the next estrus. In the second experiment, the effect of a MAP-based treatment was compared with an 11-days apart double injection prostaglandin ($PGF_{2\alpha}$) treatment for synchronization of estrus and conception rates. The estrus synchronization and conception rates obtained by $PGF_{2\alpha}$ protocol were non-significantly higher than those of MAP-based protocol (90 vs. 70% and 44.44 vs. 42.86%). The synchronization and conception rates of cyclic buffaloes (80 & 50%, respectively) receiving MAP-based regimen were also non-significantly higher from acyclic ones (50 & 33%, respectively) receiving the same protocol. In conclusion, the treatment with locally prepared MAP sponges proved effective in synchronizing estrus and comparable with prostaglandin regimen in terms of conception rates in Nili-Ravi buffaloes.

To Cite This Article: Kausar R, SA Khanum, M Hussain, T Hussain, N Ahmad, L Ahmad and NA Qureshi, 2013. Estrus synchronization and conception rates using locally prepared methylacetoxyprogesterone sponges in cyclic and acyclic Nili-Ravi buffaloes (*Bubalus bubalis*). Pak Vet J, 33(4): 433-437.

INTRODUCTION

Pakistan is the second largest country in the world with respect to buffalo population of approximately 30.8 million heads (GOP, 2011-2012) and is among the five major milk producing countries of the world (Ahmed *et al*., 2011). Buffalo is the main dairy animal in Pakistan, contributing about 61.65% of the 47.978 million tons of milk produced annually in the country. Nili-Ravi is the most important indigenous dairy breed of buffalo. However, females of this species have been conventionally agreed as poor breeders due to delayed maturity, silent estrus, low conception rate, prolonged postpartum anestrous, long calving interval and pronounced seasonality of reproduction (Perera, 2011; Mehmood *et al*., 2012). All these factors consequently lead to a shortage of milk production in the country during certain periods of the year.

The problem of poor estrus exhibition and its detection in the buffalo can be ameliorated, and reproductive efficiency improved, by various estrus synchronization strategies including progesterone releasing intra-vaginal device (PRID), progesterone sponges and prostaglandin F_2 alpha ($PGF_{2\alpha}$) protocol etc. However, the data on progesterone-based estrus synchronization regimens in buffaloes is deficient, requiring extensive studies to confirm the efficacy of progesterone induced estrus (De Rensis and Lopez-Gatius, 2007). Various analogues of progesterone may be effectively incorporated in such regimens. A recent study suggested that MAP, incorporated in estrus synchronization protocol in cyclic buffaloes, increased the follicular recruitment after its administration and speeded up follicular growth to 1.7 folds immediately after its withdrawal. Thus, MAP should also be evaluated for its utility in acyclic buffaloes (Garcia *et al.,* 2008).

The two progesterone releasing devices, PRID and Controlled Internal Drug Release (CIDR) have been used for induction of estrus in buffaloes (Presicce *et al*., 2005; Naseer *et al*., 2011). The luteolytic effect of $PGF_{2\alpha}$ is well-documented in this species (Pandey *et al.,* 2011). In cows, progestagen sponges have synchronized estrus equally successfully as the CIDR (Martinez *et al*., 2011). Sponges are cheaper, offer a precise synchronization of estrus and can be easily manufactured locally. However, information regarding the efficacy of MAP sponges in terms of the estrus synchronization and conception rates in the buffalo is limited.

In Pakistan, at present, no local made commercial progestagen-releasing intra-vaginal device is available for synchronizing estrus in buffaloes. Moreover, relatively little work has been conducted on estrus synchronization using progesterone based protocols in Nili-Ravi buffaloes in their native environment (Naseer *et al*., 2011). Therefore, the present study was planned to evaluate the efficacy of locally prepared MAP sponges for estrus synchronization and conception rate in cyclic and acyclic Nili-Ravi buffaloes.

MATERIALS AND METHODS

Sponge preparation: In this study, two experiments were performed to evaluate the estrus synchronization and conception rates by using locally prepared MAP sponges in buffaloes**.** Sponges were prepared by considering the conventional method for sheep (Robinson, 1965) with necessary modifications for buffaloes. Briefly, polyurethane sponges (diameter 6 cm & length 10 cm) were impregnated with 250 mg MAP (Depot Provera; The Pfizer, Belgium) dissolved in 20 ml ethanol for the treatment group, whereas the plain sponges for control lot (in 1st experiment) were simply washed with 20 ml ethanol and left overnight for drying. The sponges were then dusted with antibiotics powder (streptomycin/ penicillin: 5 g) just before their placement in the cranial part of the vagina of the animal. The sponges were deposited in the vagina using sterilized cylindrical glass applicators, lubricated with paraffin oil at the inserting end.

Experiment No. 1

Experimental design and animal management: The first experiment was conducted on 20 lactating buffaloes, 6-10 years of age, kept at the Livestock Farm, University of Agriculture, Faisalabad, Pakistan in October, 2010. Animals were fed green fodder (sorghum, mott grass) and concentrate (maize oil cake, 60%; corn gluten, 30%) along with mineral mixture at the time of milking. The buffaloes were inserted with MAP sponges (treated; n=10) and plain sponges (controls; n=10) for 16 days (irrespective of the stage of estrous cycle). The treated and control buffaloes were sub-grouped into cyclic (with functional corpus luteum (CL) on the ovary; n=4) and acyclic (having no functional CL on the ovary; n= 6).

Estrus detection: Buffaloes in all groups were monitored for onset of estrus using a teaser bull twice a day (in the morning and evening), up to 5 days after removal of sponges (Singh, 2003). In buffaloes showing positive reaction to the teaser, the estrus was confirmed through examination of reproductive organs per rectum. The day of estrus was specified as d-0. The presence of functional CL was confirmed in all the synchronized animals through rectal palpation on d-11 after removal of sponges.

Blood sampling: Blood sampling was started from synchronized buffaloes through jugular vein, on the d-0 of the synchronized estrus, and continued on alternate days up to 24 days. Serum was separated by centrifugation (2000xg) and stored at -20^{o}C for the estimation of serum progesterone concentrations.

Serum progesterone estimation: Serum progesterone concentrations were estimated by radioimmunoassay using commercially available kits (Immunotech, France). The sensitivity of the assay was 0.05 ngml^{-1} and the inter-assay and intra-assay coefficients of variation were 7.2 and 6.5%, respectively. The cross reactivity with other steroids ranged <0.01-15%.

Experiment No. 2.

Experimental design and animal management: The second experiment was carried out on 40 healthy multiparous buffaloes (7-12 years of age) kept at the Livestock Experiment Station (LES), Bahadarnagar, Okara, Pakistan in December, 2010. Animals were kept on berseem (*Trifolium alexandrinum*), along with the concentrate. Buffaloes were randomly divided into 2 groups. The first group, consisting of 10 cyclic and 10 acyclic animals, was inserted with MAP sponges for a period of 7 days alongwith an injection of $PGF_{2\alpha}$ (Lutalyse: 25 mg, The Pfizer, Belgium), on the day of removal of sponges. The second group also consisted of 20 cyclic buffaloes and received two injections of $PGF_{2\alpha}$ (25 mg) 11-days apart.

Artificial insemination: Estrus was detected using a teaser bull after the last injection of $PGF_{2\alpha}$, and buffaloes showing signs of true estrus were inseminated with frozen/thawed semen from bulls of known fertility (Semen Production Unit, Qadirabad, Sahiwal, Pakistan). The pregnancy diagnosis was performed 60 days later through rectal palpation.

Statistical analysis: The estrus synchronization and conception rates in cyclic and acyclic buffaloes of treated and control groups were compared by Z test for proportions (PH Stat 2, Microsoft Excel 2002) and $P<0.05$

was regarded as significant. The data regarding progesterone concentrations were analyzed in terms of standard deviation to the group means.

RESULTS

Experiment 1

Estrus synchronization: The data on estrus synchronization in cyclic and acyclic buffaloes following treatment of MAP and plain sponges are presented in Table 1. Among cyclic buffaloes, MAP treatment resulted in 75% synchronization rate, while none of the buffaloes of the control group was synchronized ($P<0.05$). Similarly, in acyclic animals, 50% were synchronized with MAP treatment, while none could be synchronized in control group ($P<0.05$). Thus, synchronization rate in both cyclic and acyclic animals treated with MAP sponges was higher compared to control. However, the difference in synchronization rate between cyclic and acyclic buffaloes of treated groups (75 vs. 50%) was non-significant. These results confirmed the efficacy of MAP sponges for synchronizing estrus in both cyclic (75%) and acyclic (50%) buffaloes.

Serum progesterone profiles during estrous cycle: The serum progesterone profiles during synchronized estrous cycles in cyclic and acyclic treated buffaloes are presented in Fig.1. At synchronized estrus, mean progesterone concentrations in cyclic and acyclic buffaloes were 0.26±0.08 and 0.32±0.27 $ngml^{-1}$, respectively. Progesterone concentrations gradually increased in next few days, reaching peak levels of 3.00±1.80 to 3.45±0.44 $ngml^{-1}$ on d-14 in the two groups. These levels were maintained up to d-16 and then a drop (2.43±0.62 to 2.79±0.40 $ngml^{-1}$) was observed on d-18 which gradually decreased to basal values (<1 $ngml^{-1}$) on the next estrus. One acyclic buffalo treated with MAP sponge showed a short estrous cycle, as was revealed by serum progesterone profile (Fig. 2). It exhibited gradually increasing levels of progesterone after estrus, reaching a peak of 3.55 $ngml^{-1}$on d-8. Then, a decreasing trend was observed from d-10 which dropped to basal level on d-14. Afterwards, progesterone profile again showed an increasing trend up to d-24. This shows the initiation of next estrus at d-14.

Experiment 2: In this trial, the comparative effects of MAP-based and $PGF_{2\alpha}$-based protocols on estrus synchronization and conception rates in Nili-Ravi buffaloes were observed and the results are expressed in Table 2. The overall estrus synchronization (90 vs. 70%) and conception rates (44.44 vs. 42.86%) for $PGF_{2\alpha}$-based protocol were non-significantly higher than the overall synchronization and conception rates obtained by MAP-based protocol. There was also non-significant difference ($P>0.05$) in estrus synchronization rate (90 vs. 80%) and conception rate (44.44 vs. 50%) between cyclic buffaloes of both treatment groups. The estrus synchronization rate of cyclic buffaloes receiving MAP-based protocol (80%) was slightly higher, but not significant, than acyclic ones receiving the same protocol (60%). Similarly, the conception rate (50 vs. 33%) of cyclic buffaloes receiving MAP-based regimen, was slightly higher than acyclic

Table 1: Estrus synchronization rate after treatment with MAP sponges in cyclic and acyclic Nili-Ravi buffaloes

Groups	Reproductive status	n	Synchronization rate	
			No.	Percent
MAP-Treated	cyclic	4	3	75^A
	Acyclic	6	3	50^A
	Total	10	6	60^A
Control	Cyclic	4	0	0^B
	Acyclic	6	0	0^B
	Total	10	0	0^B

A, B Values with different superscripts within a column differ significantly ($P<0.05$).

Table 2: Estrus synchronization and conception rate using MAP-$PGF_{2\alpha}$ regimen in comparison to double injection protocol of $PGF_{2\alpha}$ in Nili-Ravi buffaloes

Treatments	Reproductive Status	n	Estrus synchronization rate		Conception rate	
			No.	Percent	No.	Percent
MAP-$PGF_{2\alpha}$	Cyclic	10	8	80	4	50
	Acyclic	10	6	60	2	33
	Total	20	14	70	6	42.86
$PGF_{2\alpha}$(double inj.)	Cyclic	20	18	90	8	44.44

The differences in estrus synchronization rate and conception rate among various groups were non-significant

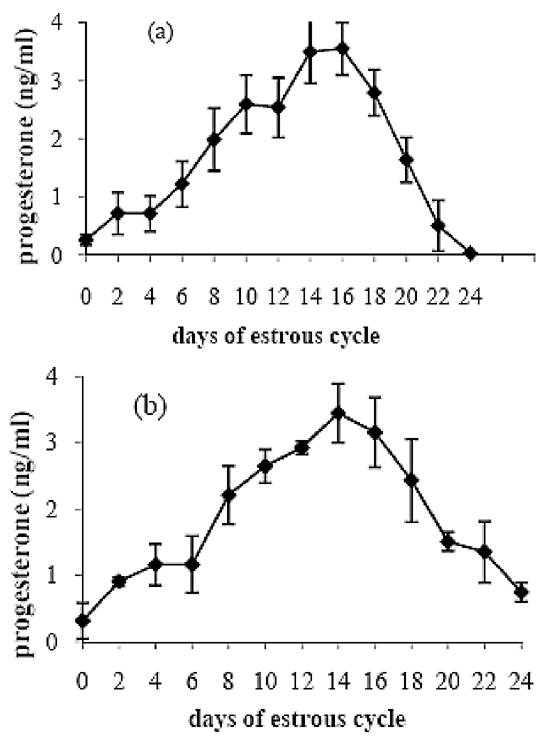

Fig. 1: Mean (±SD) serum progesterone concentrations during synchronized estrous cycles in (a) cyclic (n:3) and (b) acyclic (n:2) buffaloes.

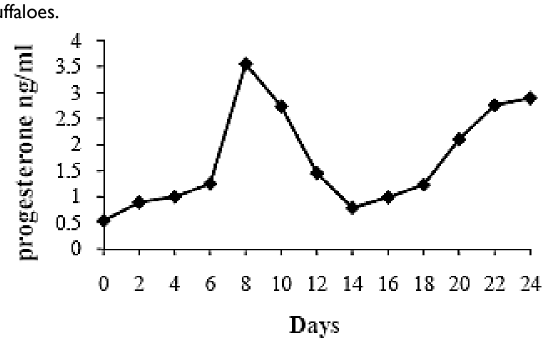

Fig. 2: Serum progesterone concentrations showing a short estrous cycle in a buffalo of acyclic group.

ones receiving the same protocol (Table 2). Thus, both the treatments proved equally effective in terms of estrus synchronization and conception rates both in cyclic and acyclic buffaloes.

DISCUSSION

Estrus synchronization rate: Commercially available intra-vaginal progestagen sponges have been used successfully to synchronize estrus in goats (Mehmood *et al*., 2011), sheep (Naderipour *et al*., 2012) and cows (Martinez *et al*., 2011). Locally manufactured MAP sponges in the present trials induced estrus in cyclic as well as acyclic buffaloes and synchronization rates by these pessaries are comparable to other intra-vaginal progesterone devices such as CIDR, which induced cyclicity in 50% acyclic buffaloes and 67% heifers (Singh, 2003). In the first experiment, the number of cyclic buffaloes was too small (4 only), giving an estrus synchronization rate of 75% (3/4); nevertheless, the 2nd experiment was executed with 10 cyclic buffaloes, giving a synchronization rate of 80% (8/10), confirming the efficacy of MAP sponges. The longer retention period (10-14 days) of CIDR in the animals induced higher estrus response (83 vs 33%) than shorter retention time (8 days). However, in the present study, MAP sponges exhibited relatively higher synchronization rates (60-80%) for a short retention period of 7 days (experiment 2), when used with the co-treatment of $PGF_{2\alpha}$, as compared to 50-75% when used alone for 16 days. Similarly, Azawi *et al.* (2012) achieved a 100% synchronization rate by a CIDR-based estrus synchronization regimen with eCG and GnRH, given at the time of removal of CIDR.

Conception rate: The conception rate for MAP-prostaglandin treatment in the present trial (42.86%) is higher than that achieved by using PRID (28%) in multiparous buffaloes (Presicce *et al*., 2004). However, the pregnancy rate was improved (70.5%), when PRID was used with PMSG (Presicce *et al*., 2005). Similarly, 80% conception rate was obtained after CIDR treatment in another study (Singh, 2003). The conception rate for MAP sponges in the current study (42.86%) is comparable with that recorded by treatment of reused CIDR and a new one (37.1% and 36.6%, respectively; Naseer *et al*., 2011). It is also in agreement with Azawi *et al*. (2012), who recorded 40% conception rate in Iraqi buffaloes by using a 7-day treatment with CIDR along with eCG and GnRH at the end of treatment. Recently, Martinez *et al*. (2011) obtained 61.5 and 57% conception rates in cow heifers by MAP sponges and CIDR, respectively.

The conception rate by double injection $PGF_{2\alpha}$ protocol in the present study (44%) is in line with the 39.3% pregnancy rate in buffaloes observed by Pandey *et al*. (2011). This pregnancy rate was correlated with the diameter of pre-ovulatory follicle (POF) in buffaloes. All the buffaloes with large follicles (>14 mm) conceived, whereas those having POF between 12 and 14 mm had a pregnancy rate of 35.3% and it was 0% for animals having POF <12 mm in diameter (Pandey *et al*., 2011).

Serum progesterone profile during estrous cycle: Serum progesterone profiles during estrous cycle recorded in the present work are almost similar to the previous reports. A meta-analysis of peripheral progesterone profiles of Murrah buffaloes revealed that during periestrus phase, peripheral progesterone was at basal level (0.30±0.06 $ngml^{-1}$), increasing to 1.94±0.03 $ngml^{-1}$ at mid-luteal phase and then declining to 1.24±0.02 $ngml^{-1}$ at late luteal phase (Mondal *et al*., 2010). This pattern of progesterone production during estrous cycle follows the changes of CL function in the ovary.

In buffaloes, the average duration of estrous cycle is 22 days, and two waves of follicular development predominantly occur during estrous cycle (Warriach and Ahmed, 2007). One of the acyclic animals showed a short estrous cycle of 14 days in the current study. In an earlier report (Baruselli *et al*., 1997), the estrous cycle of short length (13 days) was associated with the occurrence of a single wave of follicular growth and low levels of progesterone. However, pattern of follicular development could not be monitored in the buffalo with short estrous cycle in this study. In the current study, the buffalo with short estrous cycle presented a sudden rise (3.55 ng ml^{-1}) in serum progesterone on d-8 and then the values declined afterwards. Moreover, the resumption of postpartum cyclicity in buffaloes has been reported to be associated with one or more short cycles of less than 18 days (Yindee *et al*., 2007). The short cycles suggest unfavorable environmental and nutritional factors and the abnormal secretion of progesterone and estradiol (Nanda *et al*., 2003).

Conclusion: The treatment with locally prepared MAP sponges can be successfully used to synchronize estrous cycle in cyclic and acyclic Nili-Ravi buffaloes and the results are comparable with those of prostaglandin regimen in terms of achieving optimum conception rates. However, efficacy of such sponges in low breeding season and in combination with other hormones needs to be investigated before their use at commercial level.

Acknowledgement: Authors are thankful to Muhammad Younis and Riaz Hussain Virk, Faculty of Animal Husbandry, University of Agriculture, Faisalabad; and Rashid Ahmad, LES, Bahadurnagar, Okara, for providing animal keeping facilities. Authors also acknowledge Higher Education Commission, Islamabad, for funding under Indigenous Scholarship Scheme to Shahid Mehboob Rana, GC University, Faisalabad and Muhammad Ishaq and Ehsan Rashid, NIAB, Faisalabad, for their assistance in conducting these experiments.

REFERENCES

Ahmed F, H Shah, I Raza and A Saboor, 2011. Forecasting milk production in Pakistan. Pak J Agri Res, 24: 82-85.

Azawi OI, MD Ali, SA Oday, A Salih, AS Al-Hadad, SJ Mouayad and AS Abdul-Hussien, 2012. Comparative efficacy of different CIDR protocols for the treatment of postpartum anestrous in Iraqi buffaloes. Vet World, 5: 201-205.

Baruselli PS, RG Mucciolo, JA Visintin, WG Viana, RP Arruda, EH Madureira, CA Oliveira and JR Molero-Filho, 1997. Ovarian follicular dynamics during the estrous cycle in buffalo (*Bubalus bubalis*). Theriogenology, 47: 1531-1547.

De Rensis F and F Lopez-Gatius, 2007. Protocols for synchronizing estrus and ovulation in buffalo (*Bubalus bubalis*): A Review. Theriogenology, 67: 209-216.

Garcia AR, BS Nahúm, JBL Junior, NA Costa, KS Gonçalves, MYA Miyasaki, AFC Andrade and RP Arruda, 2008. The association of medroxyprogesterone to the Ovsynch protocol for the fixed time artificial insemination of cyclic buffaloes (*Bubalus bubalis*) in the Eastern Amazon. Acta Amazonica*,* 38: 369-378.

GOP, 2011-2012. Pakistan Economic Survey, Economic Affair Wing, Government of Pakistan, Islamabad, Pakistan.

Martinez MF, G de Nava, KJ Demmers, D Tutt, MR Sabarros, B Smaill, M Corti and J Juengel, 2011. Intravaginal progesterone devices in synchronization protocols for artificial insemination in beef heifers. Reprod Domes Anim, 47: 230-237.

Mehmood MU, S Mehmood and A Riaz, 2012. Superovulatory response in summer anestrus buffaloes and cattle treated with estrus synchronization protocol. J Anim Plant Sci, 22: 888-893.

Mehmood A, SMH Andrabi, M Anwar and M Rafiq, 2011. Estrus synchronization and artificial insemination in goats during low breeding season-a preliminary study. Pak Vet J, 31: 157-159.

Mondal S, K Suresh and S Nandi, 2010. Endocrine profiles of oestrous cycle in buffalo: A meta-analysis. Asian-Austr J Anim Sci, 23: 169-176.

Naderipour H, J Yadi, AG Shad and MA Sirjani, 2012. The effects of three methods of synchronization on estrus induction and hormonal profile in Kalkuhi ewes: a comparison study. Afr J Biotech, 11: 530-533.

Nanda AS, PS Brar and S Prabhakar, 2003. Enhancing reproductive performance in dairy buffalo: major constraints and achievements. Reproduction, 61(Suppl): 27-36.

Naseer Z, E Ahmad, J Singh and N Ahmad, 2011. Fertility following CIDR based synchronization regimens in anoestrous Nili-Ravi buffaloes. Reprod Domes Anim, 46: 814-817.

Pandey A, G Dhaliwal, S Ghuman and S Agarwal, 2011. Impact of pre-ovulatory follicle diameter on plasma estradiol, subsequent luteal profiles and conception rate in buffalo (*Bubalus bubalis*). Anim Reprod Sci,123: 169-174.

Perera BMAO**,** 2011. Reproductive cycles of buffalo. Anim Reprod Sci, 124: 194-199.

Presicce GA, EM Senatore, A Bella, G De Santis, VL Barile, GJ De Mauro, GM Terzano, R Stecco and A Parmeggiani, 2004. Ovarian follicular dynamics and hormonal profiles in heifers and mixed-parity Mediterranean Italian buffaloes (*Bubalus bubalis*) following an estrus synchronization protocol. Theriogenology, 61: 1343-1355.

Presicce GA, EM Senatore, G De Santis and A Bella, 2005. Follicle turnover and pregnancy rates following oestrus synchronization protocols in Mediterranean Italian buffaloes (*Bubalus bubalis*). Reprod Domes Anim, 40: 443-447.

Robinson TJ, 1965. Use of progestagen-impregnated sponges inserted intravaginally or subcutaneously for the control of the oestrous cycle in the sheep. Nature, 206: 39-41.

Singh C, 2003. Response of anestrus rural buffaloes (*Bubalus bubalis*) to intravaginal progesterone implants and PGF2alpha injection in summer. J Vet Sci, 4: 137-141.

Warriach HM and N Ahmad, 2007. Follicular waves during the oestrous cycle in Nili-Ravi buffaloes undergoing spontaneous and PGF2α-induced luteolysis. Anim Reprod Sci, 101: 332-337.

Yindee M, M Techakumphu, C Lohachit, S Sirivaidyapong, A Na-Chiangmai and B Colenbrander, 2007. Ovarian activity and sexual behavior in the postpartum Swamp buffalo (*Bubalus bubalis*). Italian J Anim Sci, 6(Suppl 2): 632-635.