Comparative efficacy of different CIDR protocols for the treatment of postpartum anestrous in Iraqi buffaloes

O I Azawi[1*], M D Ali[3], S A Oday[2], A Salih[3], A S Al-Hadad[3], S J Mouayad[3] and A S AbdulHussien[3]

1. Department of Surgery and Theriogenology, College of Veterinary Medicine, University of Mosul, Mosul, 2. Ministry of Agriculture, Livestock Resources Services General Company, Animal Research Centre, Buffalo Development Division, Mosul, 3. Ministry of Agriculture, Livestock Resources Services General Company, Baghdad, Iraq.
* Corresponding author email: azawihh@yahoo.com
Received: 01-09-2011, Accepted: 09-10-2011, Published Online: 05-01-2012
doi: 10.5455/vetworld.2012.201-205

Abstract

The aim of this study was to evaluate and compare the efficacy of different CIDR protocols including some hormonal preparations for the treatment of postpartum anestrous of Iraqi northern buffaloes. The present study was conducted on 70 anestrous buffaloes suffering from postpartum ovarian inactivity. Buffaloes were randomly assigned to three treatment groups. Buffaloes in Treatment 1 (n=20) injected on day 0 of the experiment, GnRH 250 µg and a CIDR was placed in the anterior vagina. On day 7, they were injected with 25 mg of PGF2α and on the following day (day 8) the CIDR were removed. On day 9 a second dose of GnRH (250 µg) was injected. Buffaloes in Treatment 2 (n=20) received on day 0 GnRH (250 µg) and a CIDR were placed in the anterior vagina. On day 7, CIDR were removed and injected with GnRH (250 µg). Treatment 3 (n=20) on day 0 a CIDR were placed in the anterior vagina. On day 6, they were injected with 1000 IU of eCG. CIDR were removed on day 7 and injected with GnRH (250 µg). A control group (n=10) received no treatments. Results showed that in treatment 1 (GnRH+CIDR—^{8days}—7 $PGF_2\alpha$+9 GnRH), 15 out of 20 buffaloes received treatment exhibited estrus within 69.4 ± 8.4 h after CIDR removal. The estrus induction rate was 75%. Total number of buffaloes became pregnant was 4 (26.7%). In treatment 2 (GnRH+CIDR—$^{7\ days}$—GnRH), 65% of the buffaloes received treatment exhibited estrus within 77.8 ± 5.6 h after CIDR removal. Total number of buffaloes became pregnant was 4 (26.7%). In treatment 3 (CIDR—$^{7\ days}$— 6 eCG+7 GnRH), 100% of the buffaloes received treatment exhibited estrus within 78.3 ± 11.8 h after CIDR removal. In conclusion, our results indicate that the addition of GnRH and eCG to a progesterone-based CIDR protocol substantially improves the estrus induction and pregnancy rates in postpartum anestrous buffaloes.

Key Words: Buffalo; Anestrous; CIDR; GnRH; eCG; $PGF_2\alpha$.

To cite this article : Azawi OI, Ali MD, Oday SA, Salih A, Al-Hadad AS, Mouayad SJ, AbdulHussien AS (2012) Comparative efficacy of different CIDR protocols for the treatment of postpartum anestrous in Iraqi buffaloes, *Vet. World.* 5(3):201-205, doi: 10.5455/vetworld.2012.201-205.

Introduction

Anestrous is generally defined as the state of ovarian acyclicity, reflected by complete sexual inactivity without manifestation of estrus (Wright and Malmo, 1992). Postpartum anestrous is the period after parturition during which buffalo cow do not show behavioral signs of estrus. True anestrous condition is associated with the presence of static ovaries, and even though there is follicular development, none of the ovarian follicles that start growing becomes mature enough to ovulate. As a result of this lack of follicular maturation, ovulation does not occur while anestrous is present (Moro *et al*., 1994). Field surveys on reproductive disorders revealed that anestrous was the most common single cause of infertility of buffaloes in Iraq and its incidence ranged 12-18% (Rahawy *et al*., 2006; Azawi *et al.*, 2008). Exogenous progesterone administration induces cyclicity in a substantial proportion of cows within few days of its withdrawal (Day, 2002). CIDR have been used mainly for the synchronization of ovulation and estrus (Ravikumar *et al*., 2011) but are less applied for induction of cyclicity in true acyclic buffaloes.

Various research workers (Saini *et al.*, 1986; Chohan *et al.*, 1995) have obtained satisfactory results to stimulate ovulation, and resumption of normal cyclicity of anestrous in buffaloes. Ravikumar *et al.* (2010) used CIDR-GnRH protocol in postpartum

period and found higher fertility response in the buffaloes. Employing CIDR regimes are more desirable than other that are 10 days or less in duration and which combine synchronized luteolysis with synchronized ovarian wave patterns. CIDR may be easier to insert and remove than other intravaginal devices. Justolin *et al*. (2010) used protocols consisted in CIDR insert + GnRH on day 10 and CIDR withdrawal + PGF2α on day 3 in lactating dairy cows found an improvement of conception rate. Steckler *et al*. (2002) concluded that the inclusion of exogenous progesterone such as CIDR during the interval between GnRH and PGF2a injection prevented premature estrus and increased conception rates. Although much work has been done using ovsynch plus CIDR in cattle, information on their use in buffaloes especially postpartum lactating with infertility problems is limited.

Therefore, the aim of this study was to evaluate and compare the efficacy of CIDR and some hormonal preparations for the treatment of postpartum anestrous in Iraqi northern buffaloes.

Materials and methods

Animals: The study protocol was approved by the Ministry of Agriculture, Agricultural Research Centre. The study began in April 2011 and continued until July 2011. This study was conducted on Iraqi northern buffalo cows in Nineveh province housed at smallholders' dairy farms (latitude: 36º 20´N, longitude: 43º 8´E). Postpartum lactating buffaloes included in this study had a mean age 8.18 ± 0.33 y, mean body weight 430.4 ± 19.6 kg, mean body condition score 2.88 ± 0.06 (which is a subjective, visual assessment based on rib visibility, rump-fat thickness and fat thickness around the hooks and pins). The animals were kept outdoors near the rivers for wallowing and milked twice daily. A balanced nutritional diet including green fodder and concentrate mixture were fed to these animals. Animals with a history of caesarean operation, cystic ovaries, pyometra, metritis, endometritis, lameness, abdominal disorders or other undercurrent diseases were excluded from the study on the basis of clinical examinations to remove any conflicting influence during the study period. Following data was recorded for each buffalo cow included in the present study: name of buffalo, breed, number of parturitions, obstetrical problems if present, type of last parturition, retained placenta, vaginal prolapse, uterine prolapse, abortion, number of services, and milk production.

Clinical examinations: Rectal examination of uterus, uterine tubes, ovaries and cervix was performed to each buffalo cow with smooth ovaries (without palpable corpus luteum and follicle). Any buffalo with abnormal vaginal discharge was excluded from the study. A fluid aliquot was collected from the uterine lumen for the determination of the percentage of polymorphonuclear cells. Two stained smears per sample were used and the average of the two readings of > 12% was excluded from this study (Dubuc *et al.*, 2010). All buffalo cows were characterized with postpartum anestrous of ≥ 90 days of the last parturition without any signs of estrous or pregnancy. Rectal examination was performed secondly at 10 days interval to confirm examination of all buffaloes included in this study were having smooth inactive ovaries without palpable corpus luteum and follicle (Noakes *et al.*, 2001).

Treatment protocols: Buffaloes were randomly assigned to three treatment groups. Buffaloes in Treatment 1 (n=20) injected on day 0 of the experiment, GnRH 250 µg (Cystorelin, Ceva Sante Animale, La Ballastiere-33501, Libourne Cedex, France) as the dose suggested for Iraqi buffaloes by Rahawy et al. (2006), and a CIDR was placed in the anterior vagina. On day 7, they were injected with 25 mg of PGF2α (Intervet, B.V., Boxmer, Holland) and on the following day (day 8) the CIDR were removed. On day 9 a second dose of GnRH (250 µg) was injected. Buffaloes in Treatment 2 (n=20) received on day 0 GnRH (250 µg) and a CIDR were placed in the anterior vagina. On day 7, CIDR were removed and injected with GnRH (250 µg). Treatment 3 (n=20) on day 0 a CIDR were placed in the anterior vagina. On day 6, they were injected with 1000 IU of eCG (Synncropart, Ceva Sante Animal, France) as the dose recommended by Hammam *et al.* (2009) . CIDR were removed on day 7 and injected with GnRH (250 µg). To reduce the possibility of CIDR removal by pen mates or other buffaloes, CIDR tails were clipped to be appearing on vulva lips. A control group (n=10) received no treatments. Estrus was detected on farm by expert personnel using visual estrus detection every 3 hours (8 times daily) as the method described by Senger (1994). According to Suthar and Dhami (2010) acceptance of the male is considered as the most reliable estrus indicator in buffalo as well as frequent urination, bellowing, valver swelling and clear, shiny, stringy odorless mucus discharge. All buffaloes were mated naturally at least two times after estrous detection with buffalo bulls of proven fertility. Pregnancy status was diagnosed by palpation of uterine contents 35 days after the natural mating of the 1st induced estrous.

Table-1. Pregnancy rate of buffaloes with postpartum anestrous treated using CIDR with different treatment protocols.

Treatment protocols	No. Animals	Buffaloes exhibited estrous		Buffaloes pregnant	
		No.	%	No.	%
Treatment 1 (GnRH+CIDR—8days—7 PGF2α+9 GnRH)	20	15	75	4/15	26.7[b]
Treatment 2 (GnRH+CIDR—7 days—- GnRH)	20	13	65b	4/13	26.7[b]
Treatment 3 (CIDR—-7 days— 6 eCG+7 GnRH)	20	20	100[a]	8/20	40[a]
Control (No treatment)	10	2	10[b]	0	0

a,b: Values with different superscripts within the same column are significantly different; P< 0.05.

Statistical analysis: Data were expressed as means (±SE). Pregnancy rates and percent of buffaloes exhibiting estrus among different treatment groups and control were compared using χ^2 test. The difference between means of time exhibiting estrus was tested by the analysis of variance (ANOVA) and least significance differences (LSD). The level of significance was observed at the 5% level. Statistical analyses were performed with the software (Sigma stat, Jandel scientific software V2.0 2004; Richmond, CA, USA).

Results

None of the CIDR devices were lost during the experiment. Numbers and percentages of buffaloes exhibited estrus after the various treatment protocols of CIDR and pregnancy results are shown in Table 1. In treatment 1(GnRH+CIDR—^{8days}—7 $PGF_2\alpha$+9 GnRH), 15 out of 20 buffaloes received treatment exhibited estrus within 69.4 ± 8.4 h after CIDR removal. The estrus induction rate was 75%. Total number of buffaloes became pregnant was 4 (26.7%). In treatment 2 (GnRH+CIDR—$^{7\ days}$—GnRH), 13 out of 20 buffaloes received treatment exhibited estrus within 77.8 ± 5.6 h after CIDR removal. The estrus induction rate was 65%. Total number of buffaloes became pregnant was 4 (26.7%). In treatment 3 (CIDR—$^{7\ days}$— 6 eCG+7 GnRH), 20 out of 20 buffaloes received treatment exhibited estrus within 78.3 ± 11.8 h after CIDR removal. No significant difference was observed between the three groups in time of exhibiting estrus. The estrus induction rate was 100% for buffaloes treated with CIDR—$^{7\ days}$— 6 eCG+7 GnRH protocol. Total number of buffaloes became pregnant was 8 (40%). Two of the ten control animals came into heat after 20 and 35 days from the beginning of treatment protocol. None of the animals in the control group conceived. Higher rates of estrus induction (100%; P<0.05) and conception rate (8 buffaloes out of 20) were found in buffaloes treated with CIDR—$^{7\ days}$—6 eCG+7 GnRH.

Discussion

During the last few years, several studies have been attempted to treat infertility in buffaloes by using controlled internal drug release (CIDR), hormonal treatments such as gonadotropin releasing hormone (GnRH), gonadotropins (Gn), estrogen and prostaglandin F($PGF_{2\alpha}$) (Metwelly, 2006). CIDR has been effectively used to treat anestrous buffaloes (Zaabel *et al.*, 2009). CIDR inserted intra-vaginally which are saturated with progesterone causes an increased circulatory concentration of progesterone exerted negative feedback on hypothalamus and anterior pituitary. Hence, result in favoring GnRH, FSH and LH storage. Following termination of progesterone therapy (after CIDR withdrawal by the day 7 after insertion), the rapid drop in circulatory concentration of progesterone promotes the release of GnRH as the negative feedback of progesterone was abolished, followed by FSH an LH release with subsequent resumption of ovarian cyclicity (Zerbe *et al*., 1999). Also, the increased circulatory concentration of progesterone has sensitized the hypothalamic-pituitary system (Singh, 2003). Likewise, progesterone increased hypothalamus sensitivity to estrogen with subsequent increase in the intensity of heat (Fabre-Nys and Martin, 1991; El-Wishy, 2007b). The estrus induction rate using CIDR—7 days— 6 day eCG+7 day GnRH showed that all animals in the treated group expressed estrus. This result is in agreement with Andurkar and Kadu (1995) and Ahmed *et al.* (2010). Administration of eCG probably helps complete the recovery of the hypothalamus-pituitary–gonadal axis function already stimulated by the progesterone treatment.

Our results improved this hypothesis. Previous experiments on anestrous buffaloes have shown that a

progesterone based estrus synchronization protocol, with equine chorionic gonadotropin (eCG) given at the time of progesterone withdrawal, improved the ovulation rate (Singh *et al*., 1988). Higher pregnancy rate was achieved in postpartum anestrous buffaloes using CIDR—-$^{7\ days}$— 6 day eCG+7 day GnRH protocol.

The addition of eCG to a progesterone-based protocol might improve the ovulation and pregnancy rates of anestrous buffaloes. This is in agreement with findings of Murugavel *et al*. (2009). The main action of GnRH used at the start of progesterone treatment is to synchronize emergence of a new cohort of follicles (Rhodes *et al.*, 2003). Also, second injection of GnRH at day 7 in the CIDR—-$^{7\ days}$— 6 day eCG+7 day GnRH protocol has the additional effect of inducing ovulation and the formation of a corpus luteum in a majority of cows, resulting in elevated concentrations of progesterone in anestrous buffaloes. GnRH synchronizes the development and occurrence of follicles and results in more homogenous follicular development. Also, it induces ovulation or luteinization of dominant follicle in non cyclic animals.

However the induced ovulation in non cyclic animals stimulated luteal tissue development and function resulting in the occurrence of cyclic activity (Bao *et al.*, 2003). This work has also shown that the use of CIDR together with GnRH and eCG treatment is able to induce fertile estrus in non-cycling postpartum buffaloes. This has an economic impact on buffalo production as a greater proportion of non-cycling postpartum buffaloes can be bred early. In fact, the CIDR—-$^{7\ days}$— 6 day eCG+7 day GnRH protocol treatment increased the proportion of buffalos that became cyclic within a few days from the start of the trial. Moreover, treated animals had a higher conception rate compared with controls. In conclusion, our results indicate that the addition of GnRH and eCG to a progesterone-based CIDR protocol substantially improves the estrus induction and pregnancy rates in postpartum anestrous buffaloes.

Acknowledgements

The research was financially supported by the Ministry of Agriculture, Animal Resources Service General Company (5/2/2010). The authors wish to thank buffalo farmers for their cooperation.

Conflict of interest

Authors declare that they have no conflict of interest.

References

1. Ahmed, W. M., El-Khadrawy, H. H., Abd El Hameed, A. R. and Amer, H. A. (2010) Applied investigations on ovarian inactivity in buffalo heifers. *Int J Acad Res*, 2: 26-32.
2. Andurkar, S. B., Kadu, M. S. (1995). Induction of oestrus and fertility with CIDR device and combination in non cycling buffaloes. *Indian J Anim Reprod*, 16: 81-84.
3. Azawi, O. I., Ali, A. J. and Lazim, E. H. (2008). Pathological and anatomical abnormalities affecting buffalo cows reproductive tracts in Mosul. *Iraqi J Vet Sci*, 22: 59-67.
4. Bao, G. A., Baruselli, P. S. and Marques, M. F. (2003). Pattern and manipulation of follicular development in bos indicus cattle. *Anim Reprod Sci*, 15: 307 326.
5. Chohan, K. R., Iqbal, J., Chaudhry, R. A. and Khan, A. H. (1995). Estrus response and fertility in true anoestrous buffaloes following hormonal treatment during summer. *Pak Vet J*, 15: 6-8.
6. Day, M. L. (2002). Application of the CIDR-B to Estrus Synchronization in beef cattle. Proceedings of the 2002 CHIPS Beef Breeding Management, ICN Conference, Ames, IA.
7. Dubuc, J., Duffield, T. F., Leslie, K. E., Walton, J. S. and LeBlanc, S. J. (2010). Definitions and diagnosis of postpartum endometritis in dairy cows. *J. Dairy Sci.*, 93: 5225–5233.
8. El-Wishy, A. B. (2007b). The postpartum buffalo I Endocrinological changes and uterine involution. *Anim Reprod Sci*, 97: 201-215.
9. Fabre-Nys, C. and Martin, G. B, (1991). Roles of progesterone and oestradiol in determining the temporal sequence and quantitative expression of sexual receptivity and preovulatory LH surge in the ewe. *Endocrinology*, 130: 367-379.
10. Hammam, A. M., Hegab, A.o., Scott, W. and Ibrahim, Kh. M. (2009). Improvement of fertility in Egyptian buffaloes during summer season using different protocols for estrus synchronization. *Mansoura, Vet. Med. J.*, 11: 1-12.
11. Justolin, P., Morelli, P., Reis, M., Sá Filho, O., Aragon, F., Veras, M., Soriano, S. and Vasconcelos, J. L. (2010). Effects of treatments with hCG or GnRH on serum progesterone (P4) and conception rates (CR) in lactating dairy cows submitted to timed artificial insemination (AI) or embryo transfer (ET). *J Dairy Sci* , 93: E-Suppl, 675.
12. Metwelly, K. K. (2006). Treatment of ovarian inactivity in postpartum buffalo-cows with special reference to its economic evaluation. *Assiut Vet Med J*, 52: 214-225.
13. Moro, J., Castañeda, O. G., Ruiz, F. and Román, H. (1994). Aplicación de un sistema de registro de la producción en ganader´ıas de doble propósito. INIFAP (Ed.), VII Reunión Cient´ıfica del Sector

Agropecuario y Forestal del estado de Veracruz, Veracruz, México.
14. Murugavel, K., Antoine, D., Raju, M. S. and Lopez-Gatius, F. (2009). The effect of addition of equine chorionic gonadotropin to a progesterone-based estrous synchronization protocol in buffaloes (*Bubalus bubalis*) under tropical conditions. *Theriogenology*, 71: 1120–1126.
15. Noakes, D. E., Parkinson, T. J. and England, G. C. W. (2001). Arthur's veterinary reproduction and obstetrics. W.B. Saunders press, 8th ed, pp.399–408.
16. Rahawy, M. A., Taha, M. B. and Azawi, O. I. (2006). Clinical study of anestrous in Iraqi buffaloes in Nineveh province. *Iraqi Vet J*, 20: 113-124.
17. Ravikumar, K., Asokan, S. A., Veerapandian, C., Palanisamy, A. (2010). Ovarian status serum progesterone (P4) level and conception rate in OVSYNCH plus CIDR treated postpartum buffaloes. *Tamilnadu J Vet Anim Sci*, 7: 1-5.
18. Rhodes, F. M., McDougall, S., Burke, C. R., Verkerk, G. A., and Macmillan, K. L. (2003). Treatment of Cows with an Extended Postpartum Anestrous Interval. J Dairy Sci, 86: 1876–1894.
19. Saini, M. S., Galhotra, M. M., Kaker, M. L. and Razdan, M. N. (1986). Induction of estrus and ovulation in non-cyclic buffalo (*Bubalus bubalis*) heifers with progesterone-releasing intravaginal device and pregnant mare serum gonadotrophin and their gonadotrophin profile. *Theriogenology*, 26: 749–55.
20. Senger, P. L. (1994). The estrus detection problem: new concepts, technologies, and possibilities. *J Dairy Sci.*, 77: 2745-2753.
21. Singh, C. (2003). Response of anestrus rural buffaloes (*Bubalus bubalis*) to intravaginal progesterone implant and $PGF_{2\alpha}$ injection in summer. *J Vet Sci*, 4: 137-141.
22. Singh, G., Singh, G. B., Sharma, R. D. and Nanda, A. S. (1988). Ovarian and uterine responses in relation to Norgestomet-PMSG treatment in the true anoestrous buffalo. *Anim Reprod Sci*, 16: 7 1-74.
23. Steckler, T. L., Faulkner, D. B., Nash, T. G., Dahlquist, J. M., Ireland, F. A. and Kesler, D. J.(2002). Improvement of pregnancy and rescue of embryos in beef cows. *Theriogenology*, 57: 561.
24. Suthar, V. S. and Dhami, A. J. (2010). Estrus detection methods in buffalo. *Vet. World*, 3: 94-96.
25. Wright, P. J. and Malmo, J. (1992). Pharmacologic manipulation of fertility. *Vet Clin North Am Food Anim Pract*, 8: 57–89.
26. Zaabel, S. M., Hegab, A. O., Montasser, A. E. and El-Sheikh, H. (2009). Reproductive performance of anestrous buffaloes treated with CIDR. *Anim Reprod*, 6: 460-464.
27. Zerbe, H., Gregory, C. and Grunert, E. (1999). Zur Behandlung ovariell bedingter Zyklusstorungen beim Milchrind mit Progesteron-abgebenden Vorrichtungen. *Tierarztl Umsch*, 54: 189-192.

* * * * * * * *