EVOLUTION AND PERSPECTIVES OF TIMED ARTIFICIAL INSEMINATION (TAI) PROGRAMS IN BRAZIL - A REVIEW

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ABSTRACT

Timed artificial insemination (TAI) has been applied worldwide to improve the reproductive efficiency and enhance genetic gain of buffalo herds. The control of emergence of follicular waves and of ovulation at predetermined times, without the need for estrus detection, has facilitated the management and improved the efficiency of AI programs in buffalo during the breeding and nonbreeding season in Brazil. Currently, timed ovulation induction and TAI can be performed in buffalo using GnRH or estradiol plus progesterone/progestin (P_4)-releasing devices and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). The use of TAI protocols contributed to dissemination of AI in buffalo herds, which allowed genetic improvement and, consequently, an increase in milk and beef production of this species.

Keywords: Buffaloes, Pregnancy, Progesterone, Seasonality, TAI

Artificial Insemination (AI) is one of the major biotechnologies used in domestic species as an important tool for the dissemination of superior genetic material of paternal origin. However, the use of this technique in the conventional manner (i.e., estrus detection following AI) presents two significant difficulties in buffaloes. The first is related to inefficient estrus detection due to discrete estrous behavior. The second is related to seasonal and nutritional anestrous that leads to decreased reproductive activity in the species (Baruselli *et al*., 2013).

To avoid these problems, hormonal treatments were designed to control follicular and luteal function, synchronize estrus and ovulation and, more importantly, eliminate estrus detection by artificially inseminating buffaloes on a preplanned schedule (timed artificial insemination - TAI). These schedules provide an organized approach to the enhanced use of AI, the progress of genetic gain and the improved reproductive efficiency in dairy and beef herds (Pursley *et al*., 1995; Baruselli *et al*., 2004). In buffaloes, hormonal treatments were designed to control both luteal and follicular functions, providing exciting possibilities for the synchronization of follicular growth and ovulation that can enable the use of TAI during breeding and nonbreeding season (Singh *et al*., 1988; Baruselli *et al*., 1999a; Neglia *et al*., 2003; De Rensis *et al*., 2005).

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TAI during breeding season

Baruselli *et al.* (1999b,d) developed studies to evaluate the efficiency of Ovsynch protocol (Day 0 = D0, GnRH; D7, $PGF_{2\alpha}$; D9, GnRH; TAI 16h after the 2nd GnRH) in buffalo. These experiments confirmed that buffaloes respond to hormonal treatment and that a new follicular wave emerges occurs due to ovulation of dominant follicle present at the time of the 1st GnRH. On D7, buffaloes respond to $PGF_{2\alpha}$ (luteolysis), and on D9, approximately 80% of animals experience a synchronized ovulation within 12h. Additionally, a pregnancy rate (PR) of 50% can be obtained in cycling buffaloes during the breeding season.

Moreover, satisfactory PR of approximately 40% to 60% (Neglia *et al*., 2003; Paul and Prakash, 2005; Ali and Fahmy, 2007) was achieved with Ovsynch protocol in cycling buffalo synchronized during the breeding season. Nevertheless, the PR was influenced by body condition score (BCS; high PR per AI was achieved when BCS ≥ 3.5; 1 to 5 scale), parity (primiparous had lower PR than multiparous cows) and time of the year (higher PR was observed during the breeding than in the nonbreeding season).

Aiming to evaluate new alternatives to ovulation synchronization for TAI in buffaloes during the breeding season, our research group studied the substitution of 2nd GnRH dose in the Ovsynch protocol for LH (Berber *et al.*, 2002) or for hCG (Carvalho *et al.*, 2004). No differences were observed in the PR after TAI for LH [64.2% (97/151) *vs.* 56.5% (87/154)] and hCG [50.8% (60/118) *vs.* 46.8% (44/94)] when compared to GnRH treatments. Another study evaluated the efficiency of intravaginal progesterone (P_4) device associated with the Ovsynch protocol for TAI in buffaloes (Baruselli *et al.*, 2003b). We verified that PR was similar between animal treated with Ovsynch [55.4% (36/65)] and with Ovsynch+P_4 [57.5% (61/106)].

In an attempt to reduce treatment costs, we performed a study to evaluate the efficiency of half $PGF_{2\alpha}$ dose recommended to Ovsynch protocol in buffalo (Baruselli *et al.*, 2003a). We verified that PR was not influenced by $PGF_{2\alpha}$ dose [75µg = 48.0% (60/125) *vs.* 150µg = 40.9% (56/137)]. We also observed that 88.6% (n=35) of buffaloes from ½ dose and 90.0% (n=30) from total dose presented luteolysis, which was proven by reduction in circulating P_4 concentrations, below 1ng/mL, 48h after treatment with $PGF_{2\alpha}$. According to these results, the recommended $PGF_{2\alpha}$ dose can be reduced in 50%, which reduces the costs of Ovsynch protocol for TAI in buffaloes. However, the correct size of needles and syringes, as well the precision during the administration of lower doses of $PGF_{2\alpha}$ can determine the efficiency of use of half dose of this hormone.

Carvalho *et al.*, (2007c) documented an increase in PR and birth rates with the administration of GnRH 6 days after TAI in buffaloes on Ovsynch protocol. This GnRH administration induced the formation of an accessory corpus luteum (CL) in buffaloes. The accessory CL increased the plasmatic concentrations of P_4 and resulted in a positive effect on the PR and birth rates (Campanile *et al.*, 2010; Marques *et al.*, 2012). Studies have shown that P_4 controls the function and secretion of the uterine glandular system (Spencer *et al.*, 2004), which is important for the nutrition and development of the embryo (Mann *et al.*, 1999). Therefore, the production of accessory CLs in buffalo represents an alternative method of increasing the efficiency of TAI.

However, when Ovsynch protocol was used in anestrous buffalo (without CL), results were inferior to those obtained with cyclic buffalo. Souza *et al.* (2015) verified that buffalo without a CL responded poorly to the 1st (42.0 *vs.* 89.8 %) and 2nd (52.0 *vs.* 87.8 %) GnRH treatments and this resulted in a lower PR after TAI (20.0 *vs.* 65.3 %, respectively) compared to the animals with a CL. Results of several other studies revealed a high incidence of anestrus during the nonbreeding season (spring and summer) and lower PR were reported [7.0 to 30.0 %; (Baruselli *et al.*, 1999c; Baruselli *et al.*, 2002; Baruselli *et al.*, 2003d; De Rensis *et al.*, 2005; Ali and Fahmy, 2007; Baruselli *et al.*, 2007)]. Therefore, studies were conducted in our lab to develop treatment protocols that would increase PR in buffalo submitted to TAI during seasonal anestrus.

TAI during nonbreeding season

Previous studies carried out in postpartum, anestrous cattle have shown that P_4 treatment results in an increased LH pulse frequency during and following treatment period (Rhodes *et al.*, 2002). This lead to greater follicular fluid volume and circulating concentrations of E_2 and increased numbers of LH receptors on granulosa and theca cells of pre-ovulatory follicles (Rhodes *et al.*, 2002; Rhodes *et al.*, 2003). Furthermore, a short period of elevated P_4 concentrations during the anestrous period was important for the expression of estrus and for subsequent normal luteal function (McDougall *et al.*, 1992). Therefore, it was hypothesized that exposure of anestrous buffalo to P_4 will stimulate growth and maturation of a dominant follicle by increasing LH release and the induction of LH receptors and this will result in an increased E_2 secretion and ovulation rates.

Progesterone-based protocols developed for buffalo consist of an insertion of an intravaginal P_4 device or a norgestomet ear implant and an intramuscular (IM) injection of estradiol benzoate (EB) on a random day (D0). The combination of progesterone/progestin and E_2 at the beginning of the protocol induces follicular atresia by suppressing FSH and LH release, and then synchronous emergence of a new follicular wave in response to the subsequent FSH release in cattle (reviewed by (Bó *et al*., 2003)) and buffaloes (reviewed in (Baruselli *et al*., 2007)). Nine days later (D9), the device/implant is removed and IM doses of $PGF_{2\alpha}$ and eCG are administered. 48h later (D11), ovulation is induced by the administration of GnRH and TAI is performed 16h later (Baruselli *et al*., 2003c; Carvalho *et al*., 2007b). Treatment with intravaginal P_4 devices followed by eCG at device removal was used to increase ovulation rates, CL growth rate and P_4 concentrations during the initial early diestrus following synchronized ovulation and pregnancy rates after TAI in buffalo during the nonbreeding season (Baruselli *et al*., 2013; Carvalho *et al*., 2013).

Similar pregnancy rates were obtained following TAI protocol with eCG during the breeding and nonbreeding seasons (Monteiro, 2015). Thus, administration of eCG at the time of P_4 device removal should be considered to enhance the reproductive efficiency in lactating buffalo (*Bubalus bubalis*) subjected to TAI programs during the nonbreeding season (Carvalho *et al*., 2013).

The effect of different circulating concentrations of P_4 on follicular response in seasonal anestrous buffalo cows was evaluated using the P_4/E_2 protocol (Carvalho *et al*., 2014). The authors provided evidence that devices with different P_4 release profiles resulted in similar patterns of ovarian follicular growth and ovulation, without any detrimental effects on pregnancy rate in seasonal anestrous buffalo (Carvalho *et al*., 2014).

Other studies have evaluated the use of EB to induce ovulation (rather than GnRH) in a buffalo TAI protocol (Carvalho *et al*., 2012). During the nonbreeding season, buffalo were treated with EB (24 or 36 h after P_4 device removal) or buserelin acetate (GnRH; 48h after P_4 device removal) and TAI was performed 64 h after the P_4 device removal in both groups. No differences were observed in buffaloes treated with EB at 24 or 36 h or GnRH at 48 h for the ovulatory follicle diameter (13.1±0.3 mm; 13.7±0.3 mm; 13.7±0.3 mm, respectively), ovulation rates [78.7% (37/47); 82.0% (41/50); 84.1% (37/44), respectively] or pregnancy rates [51.3% (60/117); 45.5% (51/112); 46.4% (58/125), respectively)]. In conclusion, the induction of ovulation with EB or GnRH results in similar follicular responses, ovulation and pregnancy outcomes in buffaloes synchronized for TAI during the nonbreeding season. The low cost and similar response with EB to induce ovulation (Barros *et al*., 2000; Manes *et al*., 2012) suggests that this treatment is an acceptable alternative to GnRH.

Despite the promising results obtained from the use of TAI protocols in buffalo cows (primiparous and multiparous), there are few studies involving the use of these protocols in buffalo heifers. It is known that the small diameter of the buffalo heifer's cervix can make conventional AI difficult. However, studies conducted in buffalo heifers using semen application devices designed for AI in sheep and goats, resulting in passage of the cervix with 100% effectiveness (Carvalho *et al*., 2010b). In addition, TAI in buffalo heifers during the nonbreeding season, utilizing the P_4-E_2-based protocol (D0, P_4 device+EB; D9, P_4 device removal+eCG+$PGF_{2\alpha}$; D11, GnRH and TAI 16h later) resulted in a similar ovulation rate as the Ovsynch protocol (without P_4; 78.9% *vs.* 54.5%, respectively), however, a higher PR (47.1% *vs.* 0.0%, respectively) was obtained (Carvalho *et al*., 2007a). The low PR with the Ovsynch protocol raises questions about its use in buffalo heifers during the nonbreeding season, as was reported in buffalo cows (Baruselli *et al*., 2002; Baruselli *et al*., 2003d; Baruselli *et al*., 2007). Furthermore, similar ovulation rates (92.3% *vs.* 94.1%, respectively) and PR (47.7% *vs*. 47.7%, respectively) were observed in buffalo heifers treated with intravaginal P_4 device or norgestomet ear implant during the nonbreeding season (Carvalho *et al*., 2011). Finally, as observed in buffalo cows, similar ovulation and PR were reported after the use of EB or GnRH to induce ovulation in a P_4-E_2-eCG-based TAI protocol in buffalo heifers during nonbreeding season (80.0% *vs.* 66.7% and 40.0% *vs.* 48.1%, respectively) (Carvalho *et al.*, 2010a; Nichi *et al*., 2011). The recommended protocols for TAI in buffaloes using GnRH or EB to induce ovulation are illustrated in Figure 1.

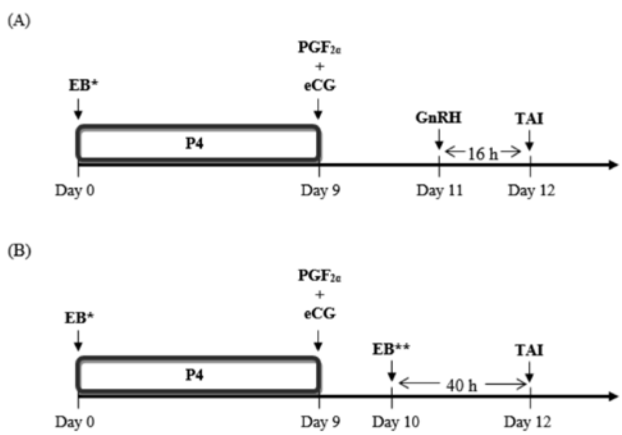

Figure 1: Schematic diagram of the P_4-E_2-based protocol with GnRH (A) or EB (B) to induce ovulation in buffaloes. EB*=2.0 mg estradiol benzoate; P_4=1.0 g progesterone; $PGF_{2\alpha}$=0.53 mg sodium cloprostenol; eCG=400 IU equine chorionic gonadotropin; GnRH=10 µg buserelin acetate or EB=1.0 mg estradiol benzoate; TAI = Timed artificial insemination**

Final considerations

Treatment protocols for TAI adapted from those used in cattle (GnRH+PGF2$_\alpha$+GnRH; P_4+EB+$PGF_{2\alpha}$+eCG/GnRH or EB) were effective in buffalo (heifers and cows) during the breeding and nonbreeding season. Currently, TAI can be used throughout the year with satisfactory ovarian responses and pregnancy outcomes. However, specific hormonal strategies to overcome seasonal anestrus are necessary and important to permit a continuous milk supply for the buffalo dairy industry.

REFERENCES

Ali, A. and Fahmy, S. (2007). Ovarian dynamics and milk progesterone concentrations in cycling and non-cycling buffalo-cows (*Bubalus bubalis*) during Ovsynch program. *Theriogenology,* **68**: 23-28.

Barros, C.M., Moreira, M.B.P., Figueiredo, R.A., Teixeira, A.B. and Trinca, L.A. (2000). Synchronization of ovulation in beef cows (*Bos indicus*) using GnRH, $PGF_{2\alpha}$ and estradiol benzoate. *Theriogenology*, **53**: 1121-1134.

Baruselli, P.S., Berber, R.C.A., Madureira, E.H. and Carvalho, N.A.T. (2003a). Half dose of prostaglandin F_{2a} is effective to induce loteolysis in the synchronization of ovulation protocol for fixed-time artificial insemination in buffalo (*Bubalus bubalins*). *Brazilian J. Vet. Res. Anim. Sci*., **40**: 397-402.

Baruselli, P.S., Bernardes, O., Braga, D., Barufi, F.B. and Tonhati, H. (2001). Factors affecting service period in dairy buffalo. In: *Proceedings of VI World Buffalo Congress*, Maracaibo, pp. 228–233.

Baruselli, P.S., Carvalho, N.A.T., Gimenes, L.U. and Crepaldi, G.A. (2007). Fixed-time artificial insemination in buffalo. *Italian J. Anim. Sci*., **6**: 107-118.

Baruselli, P.S., Carvalho, N.A.T., Henrique, C.E.P., Amaral, R., Nichi, M. and Reichert, R.H. (2003b). Use of progesterone associated with Ovsynch protocol for timed artificial insemination in buffalo (*Bubalus bubalis*), In: *Proceedings Congresso Nazionalle Sull' Allevamento del Bufalo*, pp. 265-268.

Baruselli, P.S., Carvalho, N.A.T., Henriquez, C.H.P., Amaral, R. and Nichi, M., (2002). Synchronization of ovulation for timed artificial insemination during the off breeding season in the buffalo. In: *Buffalo Symposium of Americas, 1st 2002, Belém*, Embrapa/CPATU 1, pp. 418-420.

Baruselli, P.S., Carvalho, N.A.T., Nichi, M. and Reichert, R.H. (2003c). Reduction of hCG dosage in a protocol for synchronization of ovulation for timed artificial insemination during the off breeding season in buffalo. *Bubalus bubalis* **27**: 4.

Baruselli, P.S., Madureira, E.H., Barnabe, R.C., Barnabe, J.A., Visintin, J.A., Oliveira, C.A. and Amaral, R. (1999a). Follicular dynamic during the fixed time artificial insemination protocol in buffalo. *Acta Scie. Vet*., **2**.

Baruselli, P.S., Madureira, E.H., Barnabe, V.H., Barnabe, R.C. and Berber, R.C.A. (2003d). Evaluation of synchronization of ovulation for fixed timed insemination in buffalo (*Bubalus bubalis*). *Brazilian J. Vet. Res. Anim. Sci*., **40**: 431-442.

Baruselli, P.S., Madureira, E.H., Barnabe, V.H., Barnabe, R.C., Visintin, J.A., Oliveira, C.A. and Amaral, R. (1999b). Estudo da dinâmica folicular em búfalas submetidas à sincronização da ovulação para inseminação artificial em tempo fixo. *Acta Scie. Vet*., **2**.

Baruselli, P.S., Madureira, E.H., Visintin, J.A., Barnabe, V.H., Barnabe, R.C. and Amaral, R. (1999c). Inseminação artificial em tempo fixo com sincronização da ovulação em bubalinos. *Revista Brasileira de Reprodução Animal*, **23**: 3.

Baruselli, P.S., Madureira, E.H., Visintin, J.A., Barnabe, V.H., Barnabe, R.C. and Amaral, R. (1999d). Timed insemination using synchronization of ovulation in buffalo. *Revista Brasileira de Reprodução Animal*, **23**: 360-362.

Baruselli, P.S., Marques, M.O., Nasser, L.F., Reis, E.L. and Bó, G.A. (2003e). Effect of eCG on pregnancy rates of lactating zebu beef cows treated with CIDR-B devices for timed artificial insemination. **59**: 214.

Baruselli, P.S., Reis, E.L., Marques, M.O., Nasser, L.F. and Bó, G.A. (2004). The use of hormonal treatments to improve reproductive performance of anestrous beef cattle in tropical climates. *Anim. Reprod. Sci.,* **82-83**: 479-486.

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 Bulletin* **32**: 160-176.

Berber, R.C.d.A., Madureira, E.H. and Baruselli, P.S. (2002). Comparison of two Ovsynch protocols (GnRH versus LH) for fixed timed insemination in buffalo (*Bubalus bubalis*). *Theriogenology*, **57**: 1421-1430.

Bó, G.A., Baruselli, P.S. and Martinez, M.F. (2003). Pattern and manipulation of follicular development in *Bos indicus* cattle. *Anim. Reprod. Sci.* **78**: 307-326.

Campanile, G., Baruselli, P.S., Neglia, G., Vecchio, D., Gasparrini, B., Gimenes, L.U., Zicarelli, L. and D'Occhio, M.J. (2010). Ovarian function in the buffalo and implications for embryo development and assisted reproduction. *Anim. Reprod. Sci*., **121**: 1-11.

Carvalho, N.A.T., Nagasaku, E.M., Vannucci, F.S., Toledo, L.M., Alvarez, R.H., Maio, J.R.G., Carreiro, S.S. and Baruselli, P.S. (2007a). Synchronisation of ovulation for FTAI during the off breeding season in buffalo heifer. *Acta Sci. Vet.*, **35**: 1101.

Carvalho, N.A.T., Nagasaku, E.M., Vannucci, F.S., Toledo, L.M. and Baruselli, P.S. (2007b). Ovulation and conception rate according intravaginal progesterone device and hCG or GnRH to induce ovulation in buffalo during the off breeding season. *Italian J. Anim. Sci.,* **6**: 646-648.

Carvalho, N.A.T., Soares, J.G., Porto Filho, R.M., Gimenes, L.U., Souza, D.C., Nichi, M., Sales, J.S. and Baruselli, P.S. (2013). Equine chorionic gonadotropin improves the efficacy of a timed artificial insemination protocol in buffalo during the nonbreeding season. *Theriogenology*, **79**: 423-428.

Carvalho, N.A.T., Soares, J.G., Reis, E.L. and Baruselli, P.S. (2011). Use of DIB and CRESTAR to synchronize ovulation and FTAI in buffalo heifers during the non-breeding season. *Acta Sci. Vet*., **39**: 394.

Carvalho, N.A.T., Soares, J.G., Souza, D.C. and Baruselli, P.S. (2010a). Buffalo heifers ovulation sincronization with GnRH or EB to FTAI during the off breeeding season. *Acta Sci. Vet*., **38**: 725.

Carvalho, N.A.T., Soares, J.G., Souza, D.C. and Baruselli, P.S. (2010b). Inseminação artificial em tempo fixo em novilhas bubalinas. *Agência Paulista de Tecnologia dos Agronegócios*.

Carvalho, N.A.T., Soares, J.G., Souza, D.C., Maio, J.R.G., Sales, J.N.S., Martins, B.J., Macari, R.C. and Baruselli, P.S. (2012). Ovulation synchronization with EB or GnRH in buffalo TAI during the non breeding season. *Anim. Reprod*. **9**: 523.

Carvalho, N.A.T., Soares, J.G., Souza, D.C., Vannucci, F.S., Amaral, R., Maio, J.R.G., Sales, J.N.S., Sá Filho, M.F. and Baruselli, P.S. (2014). Different circulating progesterone concentrations during synchronization of ovulation protocol did not affect ovarian follicular and pregnancy responses in seasonal anestrous buffalo cows. *Theriogenology*, **81**: 490-495.

Carvalho, N.A.T., Vannucci, F.S., Amaral, R. and Baruselli, P.S. (2007c). Use of GnRH to induce an accessory corpus luteum in buffaloes fixed time artificially inseminated. *Italian J. Anim. Sci.*, **6**: 655-658.

Cutaia, L., Tribulo, R., Moreno, D. and Bó, G.A. (2003). Pregnancy rates in lactating beef cows treated with progesterone releasing devices, estradiol benzoate and equine chorionic gonadotropin (eCG). *Theriogenology*, **59**: 216.

De Rensis, F., Ronci, G., Guarneri, P., Nguyen, B.X., Presicce, G.A., Huszenicza, G. and Scaramuzzi, R.J. (2005). Conception rate after fixed time insemination following ovsynch protocol with and without progesterone supplementation in cyclic and non-cyclic Mediterranean Italian buffaloes (*Bubalus bubalis)*. *Theriogenology*, **63**: 1824-1831.

FAO, (2016). Food and Agriculture Organization of United Nations Agriculture data, http:// http://faostat3.fao.org/browse/Q/QA/E/.

Manes, J., Aller, J.F., Callejas, S.S., Hozbor, F. and Alberio, R.H. (2012). Influence of the Length of Progestagen Treatment and the Time of Oestradiol Benzoate Application on the Ovulatory Follicle Size and Ovulation Time in Anoestrous and Cyclic Beef Cows. *Reprod. Domest. Anim*., **47**: 412-418.

Mann, G.E., Lamming, G.E., Robinson, R.S. and Wathes, D.C. (1999). The regulation of interferon-tau production and uterine hormone receptors during early pregnancy. *J. Reprod. Fert.* **54**: 12.

Marques, M.O., Nasser, L.F., Silva, R.C.P., Bó, G.A., Sales, J.N.S., Sá Filho, M.F., Reis, E.L., Binelli, M. and Baruselli, P.S. (2012). Follicular dynamics and pregnancy rates in Bos taurus x Bos indicus embryo transfer recipients treated to increase plasma progesterone concentrations. *Anim. Reprod.*, **9**: 9.

McDougall, S., Burke, C.R., Macmillan, K.L. and Williamson, N.B. (1992). The effect of pretreatment with progesterone on the oestrous response to oestradiol-17β benzoate in the postpartum dairy cow. In: *Proceeding of the New Zealand Society of Animal Production*, **52**: 4.

Monteiro, B.M. (2015). Reproductive performance of dairy buffaloes submitted to TAI protocols based on P4/E2 and eCG during breeding and nonbreeding seasons. In: *Animal Reproduction, University of Sao Paulo, São Paulo*, p. 82.

Monteiro, B.M., Souza, D.C., Vasconcellos, G.S.F.M., Corrêa, T.B., Vecchio, D., Sá Filho, M.F., Carvalho, N.A.T. and Baruselli, P.S., (2015). Ovarian responses of dairy buffalo cows to timed artificial insemination protocol, using new or used progesterone devices, during the breeding season (autumn–winter). *Anim. Sci. J.*,

Neglia, G., Gasparrini, B., Caracciolo di Brienza, V., Di Palo, R. and Zicarelli, L. (2004). First pregnancies carried to term after transfer of vitrified buffalo embryos entirely produced *in vitro*. *Vet. Res. Commun*., **1**: 233-236.

Neglia, G., Gasparrini, B., Di Palo, R., De Rosa, C., Zicarelli, L. and Campanile, G., 2003. Comparison of pregnancy rates with two estrus synchronization protocols in Italian Mediterranean Buffalo cows. *Theriogenology*, **60**: 125-133.

Nichi, M., Carvalho, N.A.T., Soares, J.G., Souza, D.C. and Baruselli, P.S. (2011). Synchronization of ovulation in water-buffalo heifers with estradiol benzoate or gonadorelin. *Acta Sci. Vet.* **39**: 391.

Paul, V. and Prakash, B.S. (2005). Efficacy of the Ovsynch protocol for synchronization of ovulation and fixed-time artificial insemination in Murrah buffaloes (*Bubalus bubalis*). *Theriogenology*, **64**: 1049-1060.

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.

Rhodes, F.M., Burke, C.R., Clark, B.A., Day, M.L. and Macmillan, K.L. (2002). Effect of treatment with progesterone and oestradiol benzoate on ovarian follicular turnover in postpartum anoestrous cows and cows which have resumed oestrous cycles. *Anim. Reprod. Sci.,* **69**: 139-150.

Rhodes, F.M., McDougall, S., Burke, C.R., Verkerk, G.A. and Macmillan, K.L. (2003). Invited review: treatment of cows with an extended postpartum anestrous interval. *J. Dairy Sci.* **86**: 1876-1894.

Sá Filho, M.F., Ayres, H., Ferreira, R.M., Marques, M.O., Reis, E.L., Silva, R.C.P., Rodrigues, C.A., Madureira, E.H., Bó, G.A. and Baruselli, P.S. (2010a). Equine chorionic gonadotropin and gonadotropin-releasing hormone enhance fertility in a norgestomet-based, timed artificial insemination protocol in suckled Nelore (Bos indicus) cows. *Theriogenology*, **73**: 651-658.

Sá Filho, M.F., Crespilho, A.M., Santos, J.E.P., Perry, G.A. and Baruselli, P.S. (2010b). Ovarian follicle diameter at timed insemination and estrous response influence likelihood of ovulation and pregnancy after estrous synchronization with progesterone or progestin-based protocols in suckled Bos indicus cows. *Anim. Reprod. Sci*., **120**: 23-30.

Sá Filho, M.F., Torres-Júnior, J.R.S., Penteado, L., Gimenes, L.U., Ferreira, R.M., Ayres, H., Castro e Paula, L.A., Sales, J.N.S. and Baruselli, P.S. (2010c). Equine chorionic gonadotropin improves the efficacy of a progestin-based fixed-time artificial insemination protocol in Nelore (Bos indicus) heifers. *Anim. Reprod. Sci*., **118**: 182-187.

Sales, J.N.S., Crepaldi, G.A., Girotto, R.W., Souza, A.H. and Baruselli, P.S. (2011). Fixed-time AI protocols replacing eCG with a single dose of FSH were less effective in stimulating follicular growth, ovulation, and fertility in suckled-anestrus Nelore beef cows. *Anim. Reprod. Sci*., **124**: 12-18.

Seren, E., Parmeggiani, A. and Campanile, E. (1995.) The control of ovulation in Italian buffalo. In: *Proceedings of the Symposium Reproduction and Animal Breeding: Advances and Strategy*, pp. 265-275.

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

Soumano, K. and Price, C.A. (1997). Ovarian follicular steroidogenic acute regulatory protein, low-density lipoprotein receptor, and cytochrome P450 side-chain cleavage messenger ribonucleic acids in cattle undergoing superovulation. *Biol. Reprod.,* **56**: 516-522.

Souza, D.C., Carvalho, N.A.T., Soares, J.G., Monteiro, B.M., Madureira, E.H. and Baruselli, P.S. (2015). Effect of the presence of corpus luteum in lactating buffaloes on the response to the Ovsynch protocol during the breeding season. In: 2*9th Annual Meeting of the Brazilian Embryo Technology Society (SBTE), Animal Reproduction, Gramado, RS, Brazil*, p. 629.

Spencer, T.E., Burghardt, R.C., Johnson, G.A. and Bazer, F.W. (2004). Conceptus signals for establishment and maintenance of pregnancy. *Anim. Reprod. Sci*., **82-83**: 537-550.

Vale, W.G., Ohashi, O.M., Sousa, J.S. and Ribeiro, H.F.L. (1990). Studies on the reproduction of water buffalo in the Amazon Valley, Brazil, Livestock Reproduction in Latin America, International Atomic Energy Agency, Vienna, pp. 201-210.

Vale, W.G., Ribeiro, H.F.L., Silva, A.O.A., Sousa, J.S., Ohashi, O.M. and Souza, H.E.M. (1996). Buffalo a non-seasonal breeder in the Amazon Valley, Brazil. In: *Proceedings of the 13th International Congress on Animal Reproduction, Sydney*, pp. 19-33.

Zicarelli, L. (1997). Reproductive seasonality in buffalo. In: *Proceedings of Third Course on Biotechnology of Reproduction in Buffaloes, Caserta, Italy*, pp. 29-52.

Zicarelli, L. (2007). Can we consider buffalo a non precocious and hypofertile species? *Italian J. Anim. Sci*., **6**: 143-154.

Zicarelli, L. (2). Enhancing reproductive performance in domestic dairy water buffalo (Bubalus bubalis), In: Lucy, M.C., Pate, J.L., Smith, M.F., Spencer, T.E. (Eds.), *Eighth International Symposium on Reproduction in Domestic Ruminants, Nottingham University Press, Anchorage, Alaska*, pp. 443-455.