

Follicular dynamics during synchronization of ovulation of nuliparous buffaloes cows during unfavourable reproductive station

C.C. Bartolomeu¹, A.J. Del Rei², C.T.G. Álvares³, G.D.Vilar⁴

¹ UAG/UFRPE Av. Bom Pastor, SN – Mundaú – Garanhuns, Brazil

² Centro Biotecnológico de Reprodução Animal da Universidade Estadual do Sudoeste da Bahia, Campus Itapetinga, Bahia, Brazil

³ DCAA-UESC, Ilheus, Bahia, Brazil

⁴ Rio Grande do Norte - Natal

Corresponding author: C.C. Bartolomeu. Av Bom Pastor, SN Mundaú Garanhuns - PE - Brazil - Tel. 008737610882 - Fax: 008737610955 - Email: c.coutinho@uag.ufrpe.br

ABSTRACT: The experiment was conducted to assess the follicular dynamics, time and synchrony of ovulation in nuliparous Murrah buffaloes (*Bubalus bubalis*) treated with an ovsynch protocol associated with progesterone. Eight nuliparous cycling buffaloes received an intravaginal CIDR device containing 1.9 g of progesterone and 100 mg of a GnRH analogue i.m. (Gonadorelin, Profertil[®]) without regard to the stage of the estrous cycle (day of treatment, day 0) followed by 500 UI eCG (eCG, Novormon[®]) 7 days later and 0.150 mg of PGF_{2α} i.m. (d-Cloprostenol, Prolise[®]) 8 days later at the time of CIDR device removal. A second-treatment of the same GnRH analogue (100 mg, i.m.) was given 48 h after PGF_{2α}. All buffaloes presented a BSC of 3,5 (scale 1 to 5, 1 = very thin and 5 = fat) Follicular dynamics was monitored by transrectal ultrasound (Pie Medical, linear transducer of 7.5 MHz). Only one female ovulated after first GnRH application. The diameter of the ovulatory follicle at the day of device removal was 7.5 ± 1.0 mm and at the day of second GnRH injection was 10.5 ± 1.2 mm. The interval between device removal and ovulation was 67.0 ± 6.0 hours and ovulation rate was 75% (6/8).

Key words: Buffalo, Estrus Synchronization, CIDR, GnRH.

INTRODUCTION - In buffalo herds, due to the lower intensity estrus expression and the seasonal pattern of reproduction, reproductive efficiency can be much more affected than in cows. Thus, estrus detection is one of the main causes of low reproductive performance in herd where reproductive biotechnologies are employed, such as artificial insemination. In these cases, the low incidence of homosexual behaviour during estrus and the great variability in the duration of estrus are aspects that limit the employment of this technology. Estrous synchronization and the synchronization of ovulation have been studied in the last years with the aim to turn this technique viable for the buffalo herds. Despite of the number of studies that have been published in the last years regarding estrus synchronization in buffaloes (de Araujo Berber et al., 2002; Baruselli and Carvalho, 2005; Resis et al., 2005), there are not many studies reporting the follicular dynamics during the hormonal

protocols and this knowledge is of great importance for the timed artificial insemination. The knowledge generated by these studies permits to evaluate the follicular responses to the treatment, turning possible to adequate the protocols once it is possible to establish the moment of the start of the follicular wave and the moment of ovulation what turns possible the establishment of the adequate hour for fixed time artificial insemination.

MATERIAL AND METHODS - This study was carried out in dairy buffalo farm located at the district of Taipu-RN, Brazil Latitude $-05^{\circ} 37' 18''$ and Longitude $35^{\circ} 35' 48''$ during the month of February. All buffaloes were kept under similar management conditions in order to minimize environment differences: Animals were kept in *Brachiara brizantha* pasture with water and mineral salt *ad libitum*. Only buffaloes characterized by good reproductive health and weighting 350 ± 28 Kg were included in the study. In addition, body condition scoring system from 1 = very thin to 5 = very fat was evaluated for each animal at the time of CIDR implant. Eight nuliparous cycling buffaloes received an intravaginal CIDR device containing 1.9 g of progesterone and 100 mg of a GnRH analogue i.m. (Gonadorelin, Profertil[®]) without regard to the stage of the estrous cycle (day of treatment, day 0) followed by 500 UI eCG (eCG, Novormon[®]) 7 days later and 0.150 mg of PGF_{2 α} i.m. (d-Cloprostenol, Prolise[®]) 8 days later at the time of CIDR device removal. A second-treatment of the same GnRH analogue (100 mg, i.m.) was given 48 h after PGF_{2 α} . All buffaloes presented a BSC of 3,5 (scale 1 to 5, 1 thin and 5 fat) Follicular dynamics was monitored by transrectal ultrasound (Pie Medical, linear transducer of 7.5 MHz) every other day from CIDR implant until CIDR removal and every 6 hour after CIDR removal until ovulation. Follicles were measured by averaging the tow largest diameter. All female buffaloes were put together with a vasectomised teaser bull fitted with a chin-ball marking harness to detec the estrus behavior.

RESULTS AND CONCLUSIONS - From the eighth buffaloes treated only one ovulated after first GnRH injection. This female buffalo presented a dominant follicle at day 0 measuring 0.95 mm. All other animals presented follicle size of 6.8 ± 0.6 mm ($X \pm S.E.M.$) that regressed after first GnRH injection. Berber et al (2002) reproted a high ovulation rate after estrus synchronization with GnRH or LH. The authors assert that the diameters of the largest follicle in animals that ovulated were larger than those of the animals that did not ovulated (9.5 ± 1.7 versus 6.7 ± 2.4 mm; $X \pm S.D.$). The diameters seen at the present experiment corroborate the reports of Berber et al (2002). Yet, according to Pursley et al (1995) the high rates of ovulation after GnRH injection are due to the presence of potentially ovulatory follicle (>9.0 mm) what happened to the animal that ovulated at the present experiment. The diameter of the ovulatory follicle at the day of device removal was 7.5 ± 1.0 mm and at the day of second GnRH injection was 10.5 ± 1.1 mm. The injection of 500UI of eCG at day 7 of treatment probably was favourable for the final growth of the ovulatory follicles. According to Baruselli et al. (2004) the addition of an eCG treatment at the time of device removal increases plasma progesterone concentration due to a bigger formed corpus luteum formed from follicle of higher diameters which is favourable for higher pregnancy rates in anestrous postpartum suckled *B. indicus* cows. As the diameter of corpus luteum is highly correlated with diameter of ovulatory follicle the employment of 500UI of eCG in the synchronization protocol is helpful to be accomplished better

pregnancy rates. The BSC is important for the success of estrus synchronization, thus, the BSC of the female buffaloes at this experiment (3,5) may have influenced positively for the follicular response observed. Azeredo et al. (2007) working with cows and heifers reported a correlation of 0.9 between pregnancy rate and BSC. The same authors describe that for each unit of increase in BSC in heifers, there is a positive effect over pregnancy rate. The interval from CIDR removal to estrus behaviour observed in this experiment (67.0 ± 6.0 hours) is very close to that reported by Porto Filho (2004) when using a similar protocol; estradiol benzoate at day 78.0 ± 12.0 and 68.0 ± 9.0 with and without eCG at the protocol, respectively. The author also reports that the eCG shortened the interval for estrus response after device removal and increases the ovulation rate. As the ovulation rate at the present experiment was very high, it can be due to the eCG as reported by Porto Filho (2004). Follicular rate of growth was higher (1.5 ± 0.1 mm/day) than the one reported by Bartolomeu (2003) 1.1 ± 0.0 mm/day. We can speculate that this fact can be due to the eCG as Bartolomeu (2003) did not use the eCG in the progesterone protocol. Regarding the diameter of the ovulatory follicle Berber et al (2002) reported that the mean diameter of the largest ovulatory follicle was 10.5 ± 1.6 mm on day seven of an Ovsynch protocol and 11.7 ± 1.4 mm on day 9 for multiparous female buffaloes during the favourable reproductive station. For the nuliparous buffaloes used in the present study the mean ovulatory follicular diameter at day 7 was 7.5 ± 1.0 mm and at the day of second GnRH injection was 10.5 ± 1.2 mm. According to Baruselli and Carvalho (2005) follicle sizes less than 0.8 mm fail to ovulate. At this experiment the one female that presented a follicle size of 0.5 mm at the day 10 of treatment did not ovulate and neither presented estrus behaviour. In conclusion, the result achieved points to the viability of fixed time artificial insemination in nuliparous buffaloes.

Table 1. Follicular dynamics during the treatment with progesterone associated to GnRH and eCG for synchronization of follicular wave and ovulation in female buffaloes.

Analysed variables	Treatment	
	Day	Event
	D0	100mg GnRH
	D7	CIDR
	D8	500 UI eCG
	D8	0.150mg of POF2a
	D10	100mg GnRH
Percentage of animals ovulating after first GnRH injection		87.5% (1/8)
Percentage of animals with start of a new follicular wave		100% (8/8)
Interval from CIDR removal to estrus behaviour		67.0 ± 6.0 (hours)
Diameter of dominant follicle at D0		7.1 ± 0.6 (mm)
Diameter of ovulatory follicle at second GnRH injection		10.5 ± 1.1 (mm)
Rate of growth of the ovulatory follicle		1.5 ± 0.1 (mm/day)

ACKNOWLEDGMENTS - To Tapuio Agropecuária.

REFERENCES - **Azeredo**, D.M., Rocha D.C., Jobim, M.I.M., Mattos, R.C., Gregory,R. M., 2007. Estrus induction and synchronization in beef heifers and their effect on the pregnancy rate and at their performance on the second reproductive season. *Ciência Rural* 37: 201-205. **Bartolomeu**, C.C. 2003. Estudo da dinâmica folicular durante o tratamento com CIDR-B e crestar visando a inseminação artificial em tempo fixo em fêmeas bubalinas (*Bubalus bubalis*). In Tese (Doutorado em Reprodução Animal) – Faculdade de Medicina veterinária e Zootecnia, Universidade de São Paulo, 158 f. **Baruselli**, P.S., Reis, E.L., Marques, M. O., Nasser, L.F., Bó, G.A., 2004. The use of hormonal treatments to improve reproductive performance of anestrus beef cattle in tropical climates. *Anim. Reprod. Sci.* 82-83: 479-486. **Baruselli**, P.S., Carvalho, N.A.T. de., 2005. Biotechnology of reproduction in buffalo (*Bubalus bubalis*). *Revista Brasileira de Reprodução Animal* 29: 4-17. **De Araújo Berber**, R.C., Madureira, E.H., Baruselli, P.S., 2002. Comparison of two Ovsynch protocols (GnRH versus LH) for fixed time insemination in buffalo (*Bubalus bubalis*). *Theriogenology* 57: 1421-1430. **Porto Filho**, R.M. Sincronização da ovulação para inseminação artificial em tempo fixo (IATF) durante a estação reprodutiva desfavorável em fêmeas bubalinas. 2004. 101 f. Tese (Doutorado em Reprodução Animal) – Faculdade de Medicina veterinária e Zootecnia, Universidade de São Paulo, São Paulo, 2004. **Pursley**, J.R., Mee, M.O., Wiltbank, M.C., 1995. Synchronization of ovulation in dairy cows using PGF_{2α} and GnRH. *Theriogenology* 44: 915-923. **Rensis**, F. De, Ronci, G., Guarneri, P., Bui Xuan Nguyen, Presicce, G.A., Huszenicza, G., 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.