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## Artificial Insemination at Fixed Time in Bufaloes

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### 1. Introduction

To maintain a calving interval of 13-14 month in buffaloes, successful breeding must take place within 85-115 days (d) after calving. Complete uterine involution and resumption of ovarian activity and heat expression usually takes place around 20-50 d post partum (dpp); therefore, there is a window of 35-95 d to rebreed a cow and get her pregnant to maintain the desired calving interval. Although artificial insemination (AI) has the potential to make a significant contribution to genetic improvement in buffaloes, its practical application has been difficult because poor estrus expression by cows and poor estrus detection by humans, a variable duration of estrus and the difficulty to predict time of ovulation. More recently, the development of protocols for synchronization of ovulation and fixed timed insemination (TAI) in buffaloes have been used to overcome these constrains and be able to use more extensively AI in commercial herds. Nevertheless, resynchronization of ovulation and TAI still remains a problem herds managed under extensive conditions for similar reasons abovementioned.

Very recently, we did four field trials to study the efficacy of different protocols that combined use of GnRH, or estradiol benzoate (EB), prostaglandin (PGF) and intravaginal progesterone (P<sub>4</sub>) releasing device (PIVD) or norgestomet ear implant (NOR) to resynchronize estrus and ovulation at day 18 post AI in buffalo cows under commercial conditions.

### 2. Materials and methods

#### 2.1 First trial

In the first field trial, we assessed with ultrasonography the ovarian follicular dynamics to study the efficacy of a combined treatment of GnRH, PGF and NOR to synchronize and resynchronize ovulations in TAI programs. Eighteen Mediterranean buffalo cows with a body condition score (BCS) of  $2.70 \pm 0.26$  (scale 1-5) from a farm in northeastern Corrientes Argentina (27° 20' 33" S and 58° 08' 27" W) were used in the study. Cows were randomly assigned to one of 3 treatments (TRT, **Figure 1**): 1) TRT1 (n=6); synchronization: day (d) -10,

8 ug GnRH (buserelin, Receptal®, Intervet SA, Argentina); d -3, 150 ug PGF (cloprostenol, Preloban®, Intervet SA, Argentina); resynchronization: d18 8 ug GnRH; d 25, 150 ug PGF; 2) TRT2 (n=6); synchronization: d -10, 8 ug GnRH and ½ ear implant for 7 days (norgestomet, Crestar®, Intervet SA, Argentina); d -3, 150 ug PGF; d -1 8 ug GnRH; resynchronization: d 18, 8 ug GnRH and ½ NOR ear implant for 7 days; d 25, 150 ug PGF; d 27 8 ug GnRH; 3) TRT3 (n=6): same protocol as TRT2 but without ear implant during synchronization and resynchronization (Figure 1). Daily ultrasounds and blood samples were taken from day -3 to day 2 during synchronization and from day 18 to day 30 during resynchronization (Figure 1). Blood samples were stored at -20 C° until P4 concentrations were analyzed by RIA (Count-A-Count®, DPC, Los Angeles, USA; intra-assay CV, 3.78%; Inter-assay CV, 9.28%).

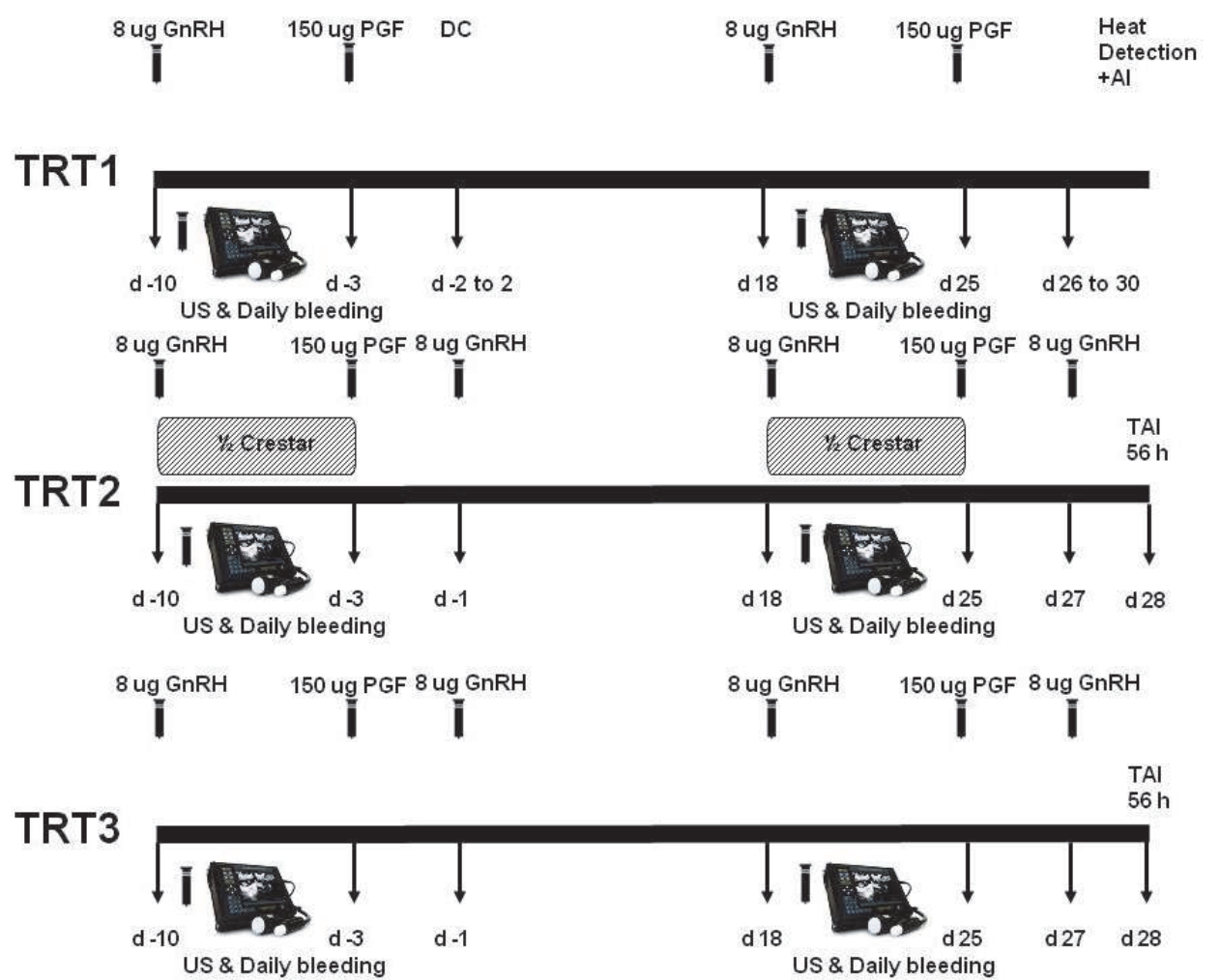


Fig. 1. Experimental design for studying follicular dynamics, time of ovulation, and fertility after synchronization and resynchronization of estrus and ovulation in buffaloes in field trials 1 and 2.

## 2.2 Results and discussion

Dominant follicle diameter prior to ovulation tended to be bigger in TRT1 compared to the TRT2 and TRT3 ( $12.58 \pm 0.67$  vs.  $10.97 \pm 0.74$  mm;  $P < 0.07$ ), and it was bigger in resynchronization compared to synchronization ( $12.56 \pm 0.46$  vs.  $10.70 \pm 0.51$  mm;  $P < 0.02$ ). On the contrary, even though the diameter of subordinate follicle was bigger with TRT3 compared to TRT1 and TRT2 ( $5.73 \pm 0.45$  vs.  $4.18 \pm 0.43$  mm;  $P < 0.02$ ), the diameter of the subordinate follicle was of equal size during synchronization and resynchronization ( $4.70 \pm 0.35$  mm). During synchronization, dominant follicle, subordinate follicle, and dominance daily growth rate was 0.55 mm/d, 0.25 mm/d and 0.75 mm/d respectively (**Figure 2**). During resynchronization, dominant follicle and dominance growth rate changed with a different pattern between treatments (**Figure 3**). Dominant follicle and dominance growth rate was bigger in TRT1 and TRT2 compared to TRT3 alone (0.87 mm/d and 0.81 mm/d vs. 0.68 mm/d; 0.65 and 0.68 mm/d vs. 0.40 mm/d; respectively;  $P < 0.01$ ; **Figure 3A and C**). In addition, during resynchronization, subordinate follicle diameter tended to increase continuously for the TRT3, whereas tended to increase and then to decrease with the other two treatments ( $P < 0.09$ ; **Figure 3B**). Even though the interval from PGF injection to ovulation was longer for TRT1 compared to the TRT2 and TRT3 groups ( $112.16 \pm 7.30$  vs.  $85.16 \pm 8.16$  mm;  $P < 0.03$ ), the interval GnRH-ovulation was equal for TRT2 and TRT3 groups ( $36.54 \pm 5.36$  vs.  $37.83 \pm 5.73$  mm;  $P > 0.37$ ). During resynchronization, a new wave started and divergence took place at day 19 and 22 for TRT1, at day 20 and 22 for TRT2, and at day 21 for TRT3. TRT2 treatment tended to be more effective in inducing follicle turnover compared to TRT1 and TRT3 alone (100% vs. 81%;  $P < 0.07$ ; **Figure 3**). During resynchronization, more dominant follicles ovulated compared to synchronization (100% vs. 81%;  $P < 0.04$ ). Lastly, even though if all 3 treatments were equally efficacious to produce follicle turnover in 90 % of cows, that efficiency tended to be higher during synchronization compared to resynchronization (100% vs. 75%;  $P < 0.07$ ).

The diameter and growth rate of the DF reported in our study agree with those reported previously by Presicce et al., (2004). They reported that in pluriparous cows, DF diameter in the first wave was  $13.3 \pm 0.5$  mm and for the second wave was  $13.8 \pm 0.6$  mm and the growth rate was  $1.6 \pm 0.1$  and  $1.3 \pm 0.1$  mm respectively. Similar results were reported very recently by Barkawi et al., (2009). In their study DF diameter for cows with 2 waves was 13 and 15 mm and for cows with 3 waves was 11, 10, and 14 mm. In our study, the DF diameter during synchronization was similar to cows with 3 waves and during resynchronization with cows of 2 waves of their study. Furthermore, the DF growth rate reported in our study is quite similar to that reported by Awasthi et al., (2007). In their study, cows with normal estrus had similar diameter and growth rate compared to our cows during synchronization but was smaller compared to our cows during resynchronization.

Progesterone concentrations previous to PGF reported by us in this study are higher than those reported previously by Chauman et al., (1983) and by Kumar et al., (1991). Maybe these higher  $P_4$  concentrations reported here are responsible for lower growth rate of DF prior to PGF administration when compared to growth rates reported previously by others (Presicce et al., 2003, 2004; Awasthi et al., 2006, 2007; Barkawi et al., 2009).

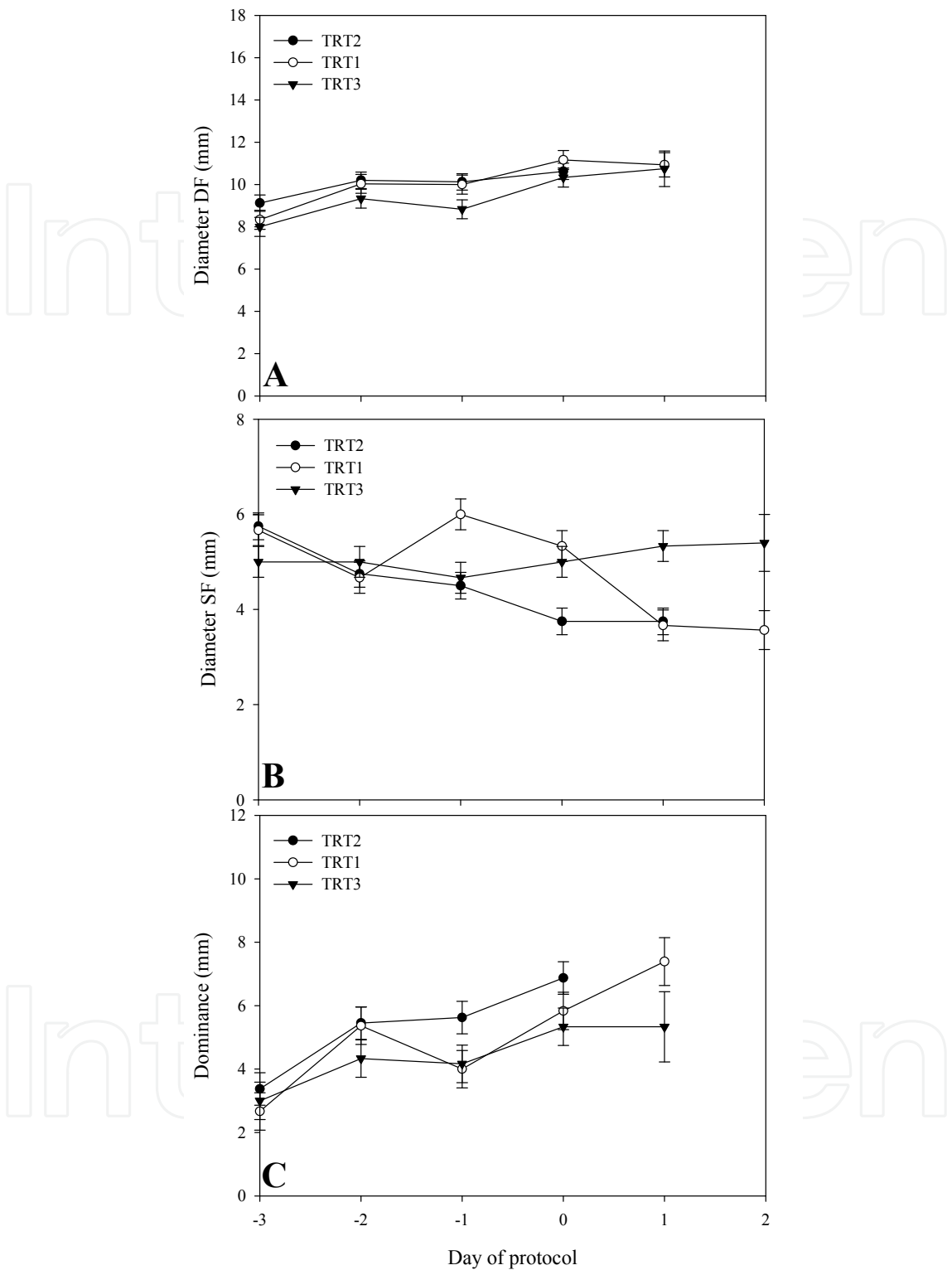


Fig. 2. Follicular dynamics by day of protocol during synchronization: diameter of the dominant follicle (A), diameter of the subordinate follicle (B), and dominance (C). TRT1: 8 ug GnRH (d-10), 150 ug PGF (d-3), heat detection every 12 h; TRT2: ½ Crestar ear implant (d-10 al -3), 8 ug de GnRH (d-10), 150 ug PGF (d-3), 8 ug GnRH (d -1); TRT3: 8 ug GnRH (d -10), 150 ug PGF (d -3), 8 ug GnRH (d -1).

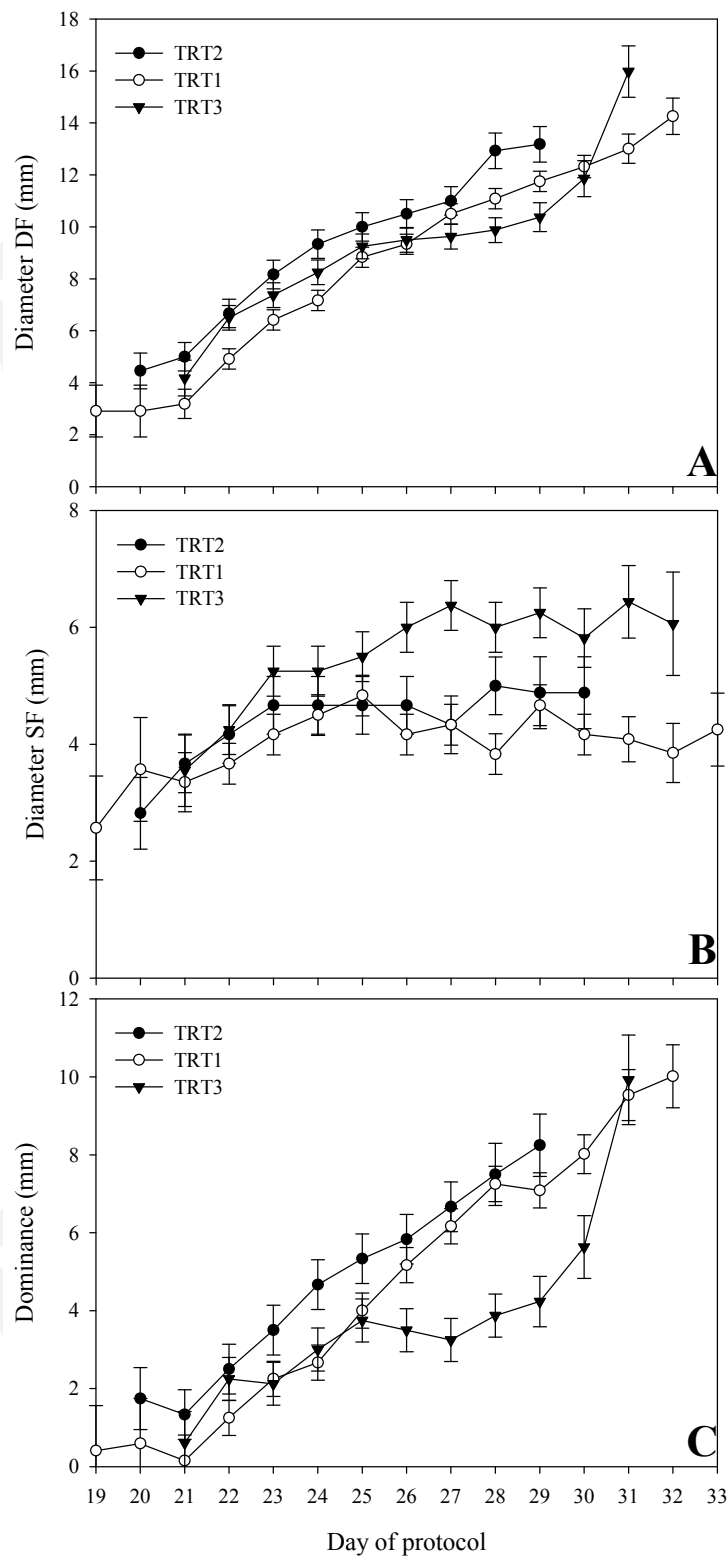


Fig. 3. Follicular dynamics by day of protocol during resynchronization: diameter of the dominant follicle (A), diameter of the subordinate follicle (B), and dominance (C). TRT1: 8 ug GnRH (d-10), 150 ug PGF (d-3), heat detection every 12 h; TRT2: 1/2 Crestar ear implant (d-10 al -3), 8 ug de GnRH (d-10), 150 ug PGF (d-3), 8 ug GnRH (d -1); TRT3: 8 ug GnRH (d -10), 150 ug PGF (d -3), 8 ug GnRH (d -1).

3. Material and methods

3.1 Second trial

In the second field trial, we assessed the fertility obtained with protocols used in the previous experiment in a commercial farm. We used 57 Mediterranean buffalo with a BCS of  $4.41 \pm 0.12$  (scale 1-5) from a farm in northeastern Corrientes Argentina ( $29^{\circ} 42' 20''$  S and  $59^{\circ} 23' 17''$  W). Cows that were randomly assigned to one of three TRT (**Figure 4**): 1) TRT1 (n=20); 2) TRT2 (n=18); 3) TRT3 (n=19).

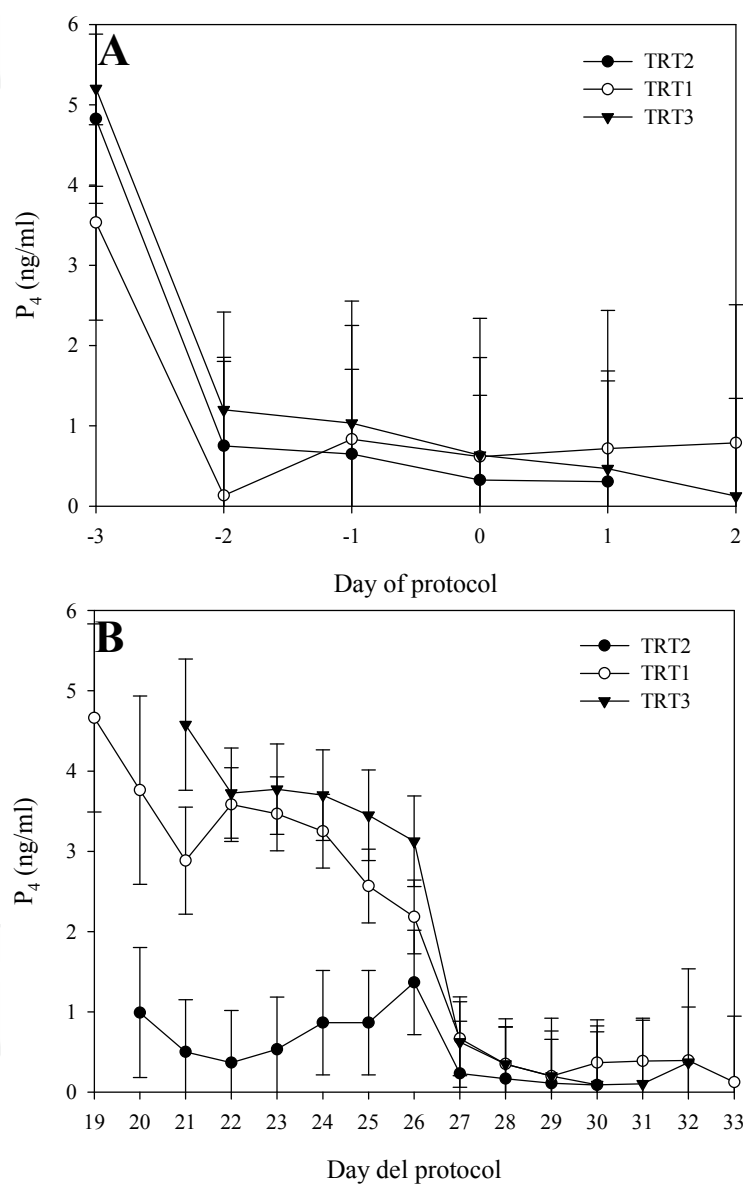


Fig. 4. Plasma P<sub>4</sub> concentrations by day of protocol during synchronization (A), and during resynchronization (B). Synchronization, TRT1: 8 ug GnRH (d-10), 150 ug PGF (d-3), heat detection every 12 h; TRT2: ½ Crestar ear implant (d-10 al -3), 8 ug GnRH (d-10), 150 ug PGF (d-3), 8 ug GnRH (d -1); and TRT3: 8 ug GnRH (d -10), 150 ug PGF (d -3), 8 ug GnRH (d -1). Resynchronization, TRT1: 8 ug GnRH (d 18), 150 ug PGF (d 25), heat detection every 12 h; TRT2: ½ Crestar ear implant (d 18 al 25), 8 ug GnRH (d 18), 150 ug PGF (d 25), 8 ug GnRH (d 27); and TRT3: 8 ug GnRH (d 18), 150 ug PGF (d 25), 8 ug GnRH (d 27).



3.2 Results and discussion

At synchronization, the percentage of cows AI was lower for the HDAI group compared to the TAI groups (80% vs. 100%,  $P<0.01$ ; **Table 1**). On the contrary, the synchronization pregnancy rate (33%), the of cow AI (97%) and percentage of cows pregnant at resynchronization (31%), the cumulative pregnancy rate for both AI (56%), the pregnancy rate for natural service (50%) and final cumulative pregnancy rate (78%) were similar between treatment groups.

	TRT1 <sup>1</sup>	TRT2 <sup>2</sup>	TRT3 <sup>3</sup>	Total
AI 1	80 (16/20) <sup>A</sup>	100 (18/18) <sup>B</sup>	100 (19/19) <sup>B</sup>	93 (53/57)
PD 1	30 (6/20)	39 (7/18)	32 (6/19)	33 (19/57)
EL 1	3	0	0	3
AI 2	91 (10/11)	100 (11/11)	100 (13/13)	97 (34/35)
PD 2	18 (2/11)	36 (4/11)	38 (5/13)	31 (11/35)
PREG IA	47 (8/17)	61 (11/18)	58 (11/19)	56 (30/54)
NS	100 (9/9)	100 (7/7)	100 (8/8)	100 (24/24)
PD 3	44 (4/9)	29 (2/7)	75 (6/8)	50 (12/24)
PREG TOT	71 (12/17)	72 (13/18)	89 (17/19)	78 (42/54)

AI: artificial insemination, PG: pregnancy diagnosis, NS: natural service, EL: embryo losses;  
A different from B,  $P<0.01$ ;  
<sup>1</sup>IACD. Synchronization: d0, 8 ug de buserelin (GnRH, Receptal®); d7, 150 mg cloprostenol (PGF, Preloban®, Intervet Argentina); d9, 8 ug GnRH; d10-12 heat detection + AI. Resynchronization: d18, 8 ug GnRH; d25, 150 ug PGF; d27 8 ug GnRH; d26-30 heat detection + AI;  
<sup>2</sup>CRE. Synchronization: d0, 8 ug GnRH + ½ norgestomet ear implant during 7 days (CRE, Crestar®, Intervet, Argentina); d7, 150 mg PGF; d9, 8 ug GnRH; d10 TAI. Resynchronization: d18, 8 ug GnRH, ½ CRE implant during 7 days; d25, 150 ug PGF; d27 8 ug GnRH; d28 TAI;  
<sup>3</sup>IATF. Synchronization: d0, 8 ug de GnRH; d7, 150 mg PGF; d9, 8 ug GnRH; d10 TAI. Resynchronization: d18, 8 ug GnRH; d25, 150 ug PGF; d27 8 ug GnRH; d28 TAI.

Table 1. Reproductive efficiency using three protocols for synchronization and resynchronization of estrus and ovulation in Mediterranean buffaloes.

4.1 Material and methods  
Third and four trial

Lastly, in the third and forth field trials, we assessed the fertility obtained with a combination of GnRH, PGF and PIVD or EB, PGF and PIVD were used to synchronize and resynchronize ovulation in TAI programs in two commercial farms.  
In the third field trial, 81 Mediterranean buffalo cows with a BCS of  $3.79\pm0.27$  (scale 1-5) from a farm in northeastern Corrientes Argentina (27° 20' 33" S and 58° 08' 27" W) were used in the study. Cows were randomly assigned to one of 2 TRT (**Figure 5**): 1) TRT1 (n=37; synchronization: d -10, 8 ug GnRH; d -3, 150 ug PGF; d -1 8 ug GnRH; d 0 TAI; resynchronization: d 18, 8 ug GnRH; d 25, ultrasound pregnancy diagnosis, open 150 ug



PGF; d 27, 8 ug GnRH; d 28 TAI), and 2) TRT2 (n=44; synchronization: d -9, 2 mg BE (BE®, Syntex, Argentina) and 1 g PIVD (Triu-B®, Biogenesis-Bagó, Argentina) for 7 days; d -2, 150 ug PGF; d -1 1 mg BE; d 0 TAI; resynchronization: d 19, 1 mg BE and 1 g PIVD for 7 days; d 26, ultrasound pregnancy diagnosis, open 150 ug PGF; d 27, 1 mg BE; d 28 TAI). Only 61 cows finished the experiment (**Table 2**). Synchronization pregnancy rate was higher in TRT2 group compared to TRT1 group (68% vs. 44%,  $P<0.03$ ). However, resynchronization pregnancy rate (78%), percent of embryonic and fetal losses (12%), less and similar result, reports by Vale et al 1989, Campanile et al 2005, 2007. The final cumulative pregnancy rate without and with embryonic and fetal losses (93% and 85%) were similar between treatments.

In the forth field trial, 119 Mediterranean buffalo cows with a BCS of  $3.17\pm0.11$  (scale 1-5) from a farm in northeastern Corrientes Argentina ( $29^{\circ} 42' 20''$  S and  $59^{\circ} 23' 17''$  W) were used in the study. Cows were randomly assigned to one of 4 TRT (**Figure 6**): 1) TRT1 (n=16); synchronization: d-10, 8 ug buserelina (GnRH, Receptal®, Intervet Argentina); d-3, 150 mg cloprostenol (PGF, Preloban®, Intervet Argentina); d-1, 8 ug GnRH; d 0 TAI; resynchronization: d18, 8 ug GnRH; d25, ultrasound pregnancy diagnosis (UPD), open cows 150 ug PGF; d27 8 ug GnRH; d 28 TAI; 2) TRT2 (n=39); synchronization: d-9, 2 mg estradiol benzoate (EB, BE®, Biogénesis, Argentina) and 1 g intravaginal P<sub>4</sub> releasing device for 7 d (PIVD, TRIU-B®, Biogénesis, Argentina); d-2, 150 mg PGF; d-1, 1 mg EB; d0 TAI; resynchronization: d19, 1 mg EB and 1 PIVD for 7 d; d26, UPD, open cows 150 ug PGF; d27 1 mg EB; d 29 TAI; 3) TRT3 (n=44); synchronization: d-10, 8 ug GnRH and 1 PDIV for 7 d; d-3, 150 mg PGF; d-1, 8 ug GnRH; d0 TAI; resynchronization: d18, 8 ug GnRH and 1 PIVD for 7 d; d25, UPD, open cows 150 ug PGF; d27 8 ug GnRH; d 28 TAI; and 4) TRT4 (n=20); synchronization: d-9, 2 mg de EB and 1 PIVD for 7 d; d-2, 150 mg PGF; d-1, 1 mg EB; d0 TAI; resynchronization: d19, 1 mg EB y 1 PIVD for 7 d; d26, UPD, open cows 150 ug PGF; d27 1 mg EB; d 28-32 AI detected heat.

#### 4.2 Results and discussion

Only 104 cows finished the experiment (**Table 3**). Even though the synchronization pregnancy rate was similar between treatments (41%), more cows were resynchronized with the TAI protocols than with the HDAI protocol (100% vs. 67%,  $P<0.01$ ). On the contrary, resynchronization pregnancy rate (57%), pregnancy rate to AI (76%), natural service pregnancy rate (30%), and final cumulative pregnancy rate (85%) were similar between treatments ( $P>0.13$ ).

De Araujo Berber et al., (2002) and Ronci and De Rensis (2005) using a GnRH + PGF + GnRH + TAI protocol (Ovsynch) obtained higher pregnancy rates than those achieve by us in these field trials. The findings could be explained because they used weaned cows and most likely all were cycling. Conversely, Paul and Prakash (2005) and Warriach et al., (2008) reported lower pregnancy rates using an Ovsynch protocol. When De Rensis and Ronci, (2005) supplemented the Ovsynch protocol with P<sub>4</sub>, pregnancy rates were similar to those obtained in our Ovsynch protocols that were supplemented with P<sub>4</sub>. Presicce et al., (2005) using a protocol that combined a PIVD with EB and PMSG obtained higher pregnancy rates compared with an Ovsynch protocol alone, but this higher pregnancy rate is more likely due to the use of PMSG than EB.

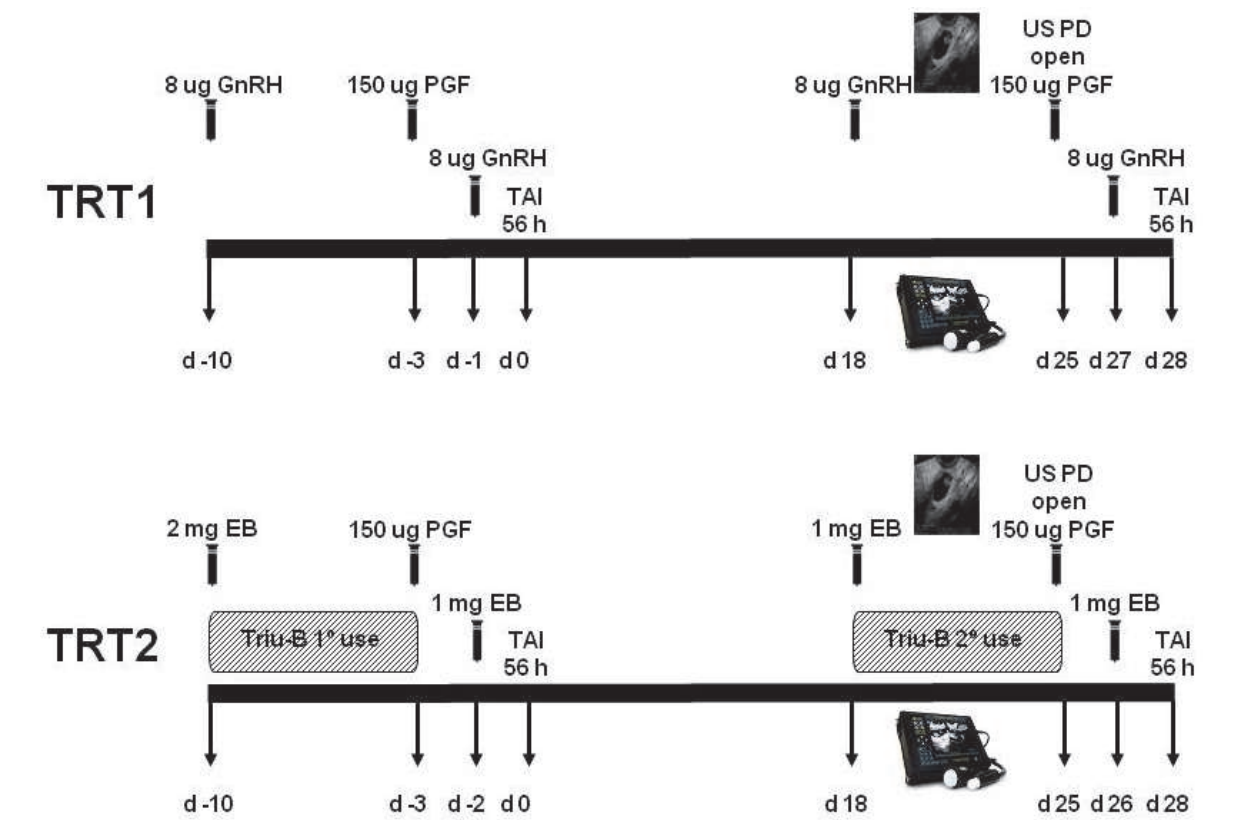


Fig. 5. Experimental design for studying fertility after synchronization and resynchronization of estrus and ovulation in buffaloes in field trial 3.

	TRT1 <sup>1</sup>	TRT2 <sup>2</sup>	Total
AI 1	37	44	81
NPD 1	3	3	6
PD 1	44% (15/34) <sup>A</sup>	68% (28/41) <sup>B</sup>	57% (43/75)
NAI 2	6	3	9
NPD 3	5		5
PD2	75% (6/8)	80% (8/10)	78% (14/18)
PREG AI	91% (21/23)	95% (36/38)	93% (57/61)
EL 50	13% (2/15)	11% (3/28)	12% (5/43)
PREG Final	83% (19/23)	87% (33/38)	85% (52/61)

PD: pregnancy diagnosis, EL: Embryo losses, NPD1: did not come to PD1, NAI2: did not come to AI2, NPD2: did not come to PD2;  
A different from B, P<0.03;  
<sup>1</sup>TRT1. Synchronization: d-10, 8 ug de buserelina (GnRH, Receptal®, Intervet Argentina); d-3, 150 mg cloprostenol (PGF, Preloban®, Intervet Argentina); d-1, 8 ug GnRH; d 0 TAI. Resynchronization: d18, 8 ug GnRH; d25, ultrasound pregnancy diagnosis, open cows 150 ug PGF; d27 8 ug GnRH; d 28 TAI.  
<sup>2</sup>TRT2. Synchronization: d-9, 2 mg estradiol benzoate (EB, BE®, Biogénesis, Argentina) and 1 g P<sub>4</sub> intravaginal releasing device for 7 d (PIVD, TRIU-B®, Biogénesis, Argentina); d-2, 150 mg PGF; d-1, 1 mg EB; d0 TAI. Resynchronization: d19, 1 mg EB y 1 PIVD for 7 d; d26, ultrasound pregnancy diagnosis, open cows 150 ug PGF; d27 1 mg EB; d 28 TAI.

Table 2. Reproductive efficiency using two protocols for synchronization and resynchronization of estrus and ovulation in Mediterranean buffaloes.

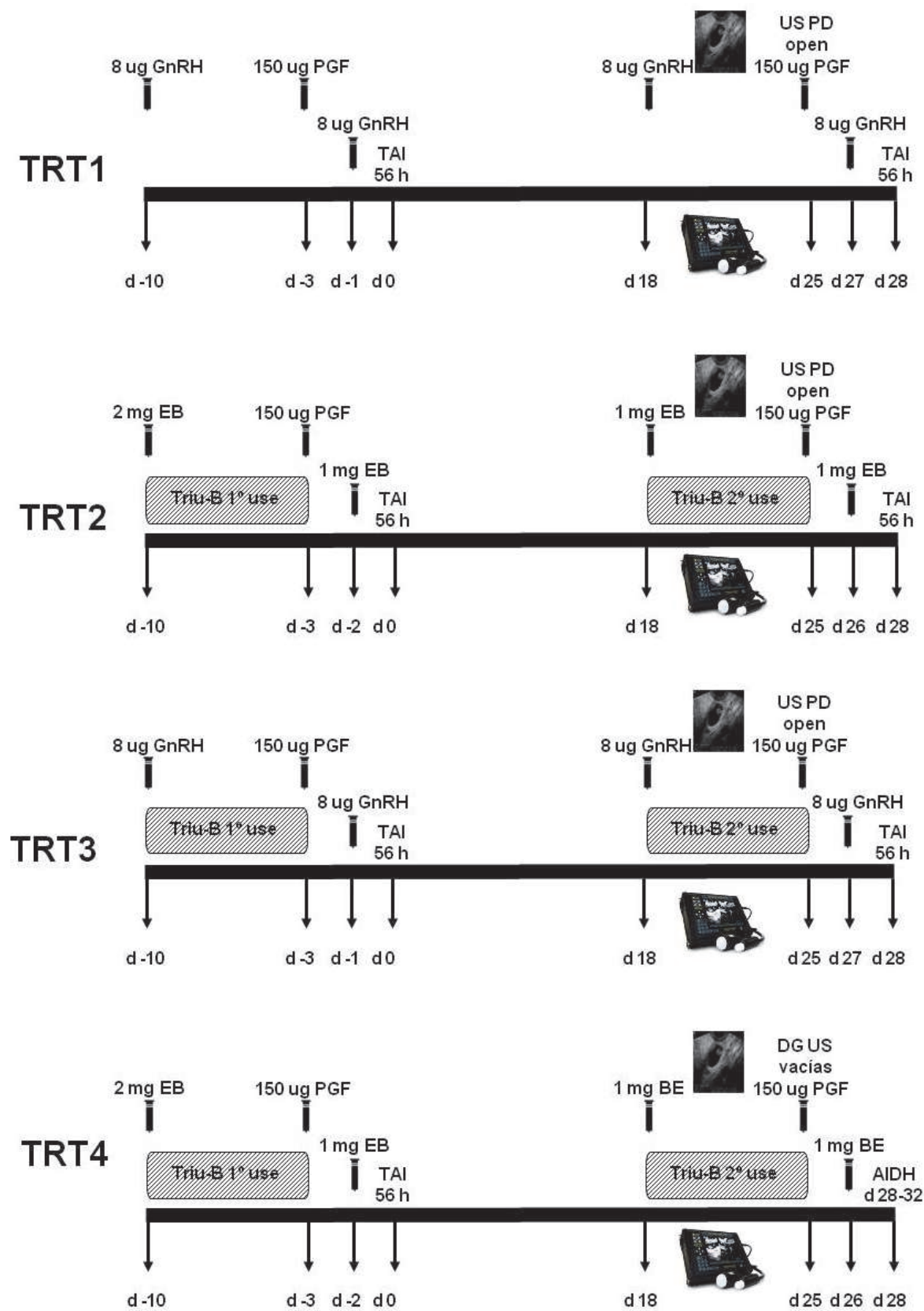


Fig. 6. Experimental design for studying fertility after synchronization and resynchronization of estrus and ovulation in buffaloes in field trial 4.

	TRT1	TRT2	TRT3	TRT4	Total
SYN	100 (16/16)	100 (39/39)	100 (44/44)	100 (20/20)	100 (119/119)
PD1	38 (6/16)	36 (14/39)	48 (21/44)	40 (8/20)	41 (49/119)
NAI2	2	5	3	0	10
RESYN	100 (8/8) <sup>A</sup>	100 (20/20) <sup>A</sup>	100 (20/20) <sup>A</sup>	67 (8/12) <sup>B</sup>	93 (56/60)
PD2	88 (7/8)	45 (9/20)	65 (13/20)	42 (5/12)	57 (34/60)
PREGAI	93 (13/14)	68 (23/34)	83 (34/41)	65 (13/20)	76 (85/109)
NS	100 (1/1)	100 (9/9)	100 (6/6)	100 (7/7)	100 (23/23)
PD3	0 (0/1)	22 (2/9)	33 (2/6)	43 (3/7)	30 (7/23)
NPD3	0	4	1	0	5
PREGT	93 (13/14)	77 (23/30)	90 (36/40)	80 (16/20)	85 (88/104)

SYN: synchronization, RESYN: resynchronization, PD: pregnancy diagnosis, NAI2: did not come to resynchronization, NPD3: did not come to PD3;  
A different from B, P<0.0001;  
<sup>1</sup>TRT1 Synchronization: d-10, 8 ug buserelina (GnRH, Receptal®, Intervet Argentina); d-3, 150 mg cloprostenol (PGF, Preloban®, Intervet Argentina); d-1, 8 ug GnRH; d 0 TAI. Resynchronization: d18, 8 ug GnRH; d25, ultrasound pregnancy diagnosis (UPD), open cows 150 ug PGF; d27 8 ug GnRH; d 28 TAI;  
<sup>2</sup>TRT2 Synchronization: d-9, 2 mg estradiol benzoate (EB, BE®, Biogénesis, Argentina) and 1 g intravaginal P<sub>4</sub> releasing device for 7 d (PIVD, TRIU-B®, Biogénesis, Argentina); d-2, 150 mg PGF; d-1, 1 mg EB; d0 TAI. Resynchronization: d19, 1 mg EB and 1 PIVD for 7 d; d26, UPD, open cows 150 ug PGF; d27 1 mg EB; d 29 TAI;  
<sup>3</sup>TRT3 Synchronization: d-10, 8 ug GnRH and 1 PDIV for 7 d; d-3, 150 mg PGF; d-1, 8 ug GnRH; d0 TAI. Resynchronization: d18, 8 ug GnRH and 1 PIVD for 7 d; d25, UPD, open cows 150 ug PGF; d27 8 ug GnRH; d 28 TAI;  
<sup>4</sup>TRT4 Synchronization: d-9, 2 mg de EB and 1 PIVD for 7 d; d-2, 150 mg PGF; d-1, 1 mg EB; d0 TAI. Resynchronization: d19, 1 mg EB y 1 PIVD for 7 d; d26, UPD, open cows 150 ug PGF; d27 1 mg EB; d 28-32 AI detected heat.

Table 3. Reproductive efficiency using two protocols for synchronization and resynchronization of estrus and ovulation in Mediterranean buffaloes.

5. Conclusion

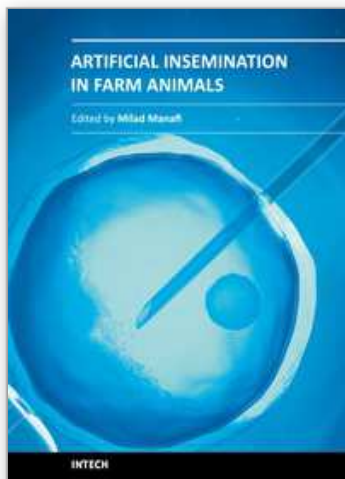
We can conclude from this series of field trials that the combination of GnRH, PGF and P<sub>4</sub> IVD or EB, PGF and P<sub>4</sub> IVD proved to be efficacious to synchronize and resynchronize ovulation in unweaned buffalo cows. Results from this work, show that a 75% pregnancy rate can be achieved during the first 28 days of the breeding season without heat detection and already taking into account early embryonic and fetal losses. Lastly, it is worth to point out that pregnancy rate achieved in all experiments with TAI protocols was numerically higher than that achieved with HDAl; hence these results indicate that TAI may be a very promising tool for genetic improvement in buffalo herds.

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## **Artificial Insemination in Farm Animals**

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Artificial insemination is used instead of natural mating for reproduction purposes and its chief priority is that the desirable characteristics of a bull or other male livestock animal can be passed on more quickly and to more progeny than if that animal is mated with females in a natural fashion. This book contains under one cover 16 chapters of concise, up-to-date information on artificial insemination in buffalos, ewes, pigs, swine, sheep, goats, pigs and dogs. Cryopreservation effect on sperm quality and fertility, new method and diagnostic test in semen analysis, management factors affecting fertility after cervical insemination, factors of non-infectious nature affecting the fertility, fatty acids effects on reproductive performance of ruminants, particularities of bovine artificial insemination, sperm preparation techniques and reproductive endocrinology diseases are described. This book will explain the advantages and disadvantages of using AI, the various methodologies used in different species, and how AI can be used to improve reproductive efficiency in farm animals.

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