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PREGNANCY RATE IN EGYPTIAN BUFFALOES AFTER SYNCHRONIZATION OF ESTRUS WITH $PGF_{2\alpha}$ OR OVULATION BY OVSYNCH PROGRAM

(With 2 Tables and 2 Figures)

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تقييم معدل الحمل فى الجاموس المصرى فى فترة مابعد الولادة باستخدام الحقن المزدوج للبروستاجلاندين أو نظام تزامن التبويض

فوزى الزهيرى ، عبد الخالق نصرة ، صلاح الغندور

تحول استطالة الفترة بين الولادتين فى ا لجاموس المصرى من زيادة الكفاءة الانتاجية لـــه مما يؤثرسلبا على اقتصاديات انتاجه الأمر الذى حدى بالبحث عن وسائل متعـــددة لزيـــادة الخصوبة به وعلية تم اختيار عدد ٤٨ جاموسة حلابة، قسـمت الـــى ثـــلاث مجموعـــات: المجموعة الأولى: حقنت فى العضل بهرمون البروستاجلاندين مرتين بينهم ١٤ يوم، ثم تـــم حقن الحيوانات التى لم تأتى فى الشياع بجرعة ثالثة ثم التلقيح الاصطناعى بعـــد +٧٢-٨٠ ساعة. المجموعة الثانية: استخدم معها الحقن المحرر للحاثة المنسلية- البروســـتاجلاندين - المحرر للحاثة أما المجموعة الثالثة فكانت عينة ضابطة. ولقد كانت نتائج المجموعة الثانيـــة أعلى فى نسبة الحمل من المجموعتين الأخرتين.

SUMMARY

Postpartum in buffaloes plays a great role in delay of the cyclicity in buffaloes that seemed to be anestrous. So, the use of hormones to induct estrus or induct ovulation has an importance to increase pregnancy rate. The present study was designed to evaluate the efficacy of the two methods in inducing high pregnancy rate. The experimental animals (48 lactating buffaloes) were divided into three groups: $PGF_{2\alpha}$ group 1(n=16); Ovsynch group 2 (n=16) and control group (n=16). Pregnancy rate was significantly ($p<0.001$)increased in group 2 than group 1 and control group due to higher level of progesterone and more accurate synchronization for ovulation., It is more suitable to use the regimen of ovsynch in postpartum buffaloes especially in multiparous females with good condition score.

Key words: *Pregnancy, buffaloes, synchronization, ovsynch program*

INTRODUCTION

Methods of synchronization of estrus have been developed in many dairy herds to manage reproduction efficiency. Management of artificial insemination is more efficient when synchronization scheme was used to reduce the variation of first heat and subsequent improving the conception of AI (King *et al.*, 1982). Synchronization protocols approved some hormones for lactating dairy cows, $PGF_{2\alpha}$ was the main one (Lucy *et al.*, 1986, Stevenson *et al.*, 1987 and Archbald *et al.*, 1992) where, $PGF_{2\alpha}$ treatment do not affect the growth of follicles but only act as luteolytic for the corpus luteum (CL), in addition to longevity heat detection after administration (Momont and Seguin, 1983; Lucy *et al.*, 1986 and Larson and Ball, 1992). These lead to low pregnancy rate (Stevenson *et al.*, 1987; Stevenson *et al.*, 1989).

Recently, another mean for induction of heat was ovsynch program that developed to synchronize ovulation in lactating dairy cows using gonadotrophin-releasing hormone (GnRH) and $PGF_{2\alpha}$ and blind insemination without detection of estrus (Pursley *et al.*, 1995). Pregnancy rate in timed AI with ovsynch was similar to AI with observed estrus (Pursley *et al.*, 1997a and b; Britt and Gaska, 1998; Stevenson *et al.*, 1999 and Stevenson *et al.*, 2000). Buffaloes show peculiarities in limited homosexual behavior during estrus, poor accuracy in estrus detection which differ than cattle. Therefore (Baruselli *et al.*, 2000; Baruselli *et al.*, 2001 and 2003) employed synchronization programs that are based either on GnRH and/or $PGF_{2\alpha}$ in female buffaloes.

So, the aim of the present study was to evaluate pregnancy rate obtained by either synchronization with $PGF_{2\alpha}$ or by ovsynch protocol in primiparous and multiparous female buffaloes.

MATERIALS and METHODS

A-Animals:

This study was conducted on 48 normal buffaloes (24 primiparous and 24 multiparous) ranging from 60 to 90 days postpartum during the breeding season from October 2005 to March 2006 in a private farm, located in EL-Behera province. The animals were with body condition score 3.0 - 3.5 (scale 1= very thin and scale 5 = very fat), weighting range from 450 to 550 Kg and their age ranged from 3 to 8 years.

Animals were fed a ration consisting of concentrates (maize grain, lean seed cake, wheat bran), berseem (Trifolium alexandrium) according to the system of nutrition in Egypt.

B-Experimental Design:

Animals were divided into three experimental groups:

1. Group I [$PGF_{2\alpha}$ group (8 primiparous and 8 multiparous)]:

Animals received two intramuscular (i.m) injections of synthetic $PGF_{2\alpha}$ α (Luprostiol 15 mg, Prosolvin, Intervet, Holland) in between 14 days apart to act well on complete mature corpus luteum (day 0 = day of first injection), then inseminated according to the a.m – p.m rule following detected heat, all animals that were not detected in heat were re-injected i.m once again with $PGF_{2\alpha}$ on day 14 from the previous injection then artificially inseminated blindly at 72 to 80 h after the third injection of $PGF_{2\alpha}$ (Figure 1) as recorded by Nebel and Jobst, 1998 and Stevenson, 2001.

2. Group II [Ovsynch group (8 primiparous and 8 multiparous)]:

Animals received GnRH agonist (Buserelin) in dose of 12 µg (Receptal, Intervet, Holland) i.m injection on day 0 (day 0 = day of first injection), then followed by injection of 15 mg $PGF_{2\alpha}$ on the 7th day. Later a second administration of GnRH on the 9th day with the same dose mentioned above followed by fixed time A.I at 16 – 20 h (Figure 2).

3. Group III, Control group (8 primiparous and 8 multiparous):

These animals didn't receive any treatment and were inseminated at a correct time after observed heat.

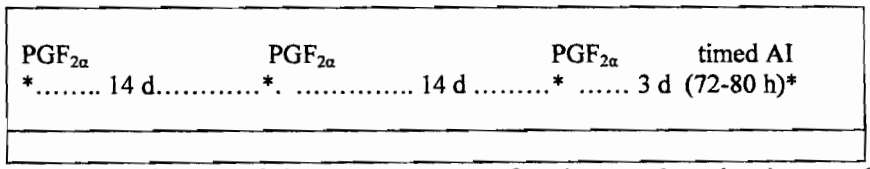


Fig. 1: Description of the program used for the synchronization method $PGF_{2\alpha}$ - $PGF_{2\alpha}$

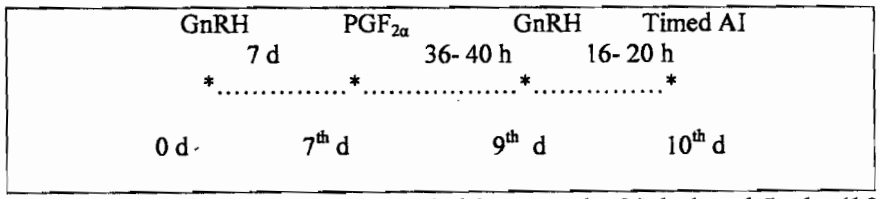


Fig. 2: Ovsynch progran as recorded in cows by Nebel and Josbt (1998) and Baruselli *et al.*, (2001) and (2003)

C-Blood Samples:

Blood samples were collected on days 0 and 25 after insemination from the jugular vein by venipuncture into 10 ml vacutainer tubes. Serum was separated and stored at –20 °C until Progesterone hormone assay.

D- Hormonal assay:

Progesterone was measured by direct radio immunoassay (RIA) using coat A count kit (Diagnostic Products Corporation) DPC.

E- Pregnancy diagnosis:

Progesterone >1.0 ng/ml on day 25 post insemination was considered as pregnant and confirmed 60 days after the insemination by rectal palpation.

F- Statistical analysis:

The data were statistically analyzed according to Snedecor and Cochran (1980). A Chi- square test was used to compare pregnancy rates to TAI (ovsynch) versus induced estrus with $PGF_{2\alpha}$.

RESULTS

Group I ($PGF_{2\alpha}$ group), 4/8(50%) primiparous buffaloes responded and became pregnant (2, 1 and 1 pregnant buffalo after the 1st, 2nd and 3rd injections respectively), 5/8 (62.5%) multiparous buffaloes became pregnant (2, 2 and 1 after the 1st, 2nd and 3rd injections, respectively) with high significance ($P < 0.001$) as shown in Table 1.

On the other hand pregnancy rates in (Group II, Ovsynch group), were 4/8 (50%) primiparous buffaloes (1 and 3 after injection of $PGF_{2\alpha}$ and 2nd injection of GnRH, respectively), 6/8 (75%) multiparous buffaloes became pregnant (1, 2 and 3 after injection of 1st injection of GnRH, $PGF_{2\alpha}$ injection and 2nd injection of GnRH, respectively) with high significant ($P< 0.001$).

Group III (control group) for primiparous and multiparous buffaloes showed pregnancy rate 3/8 (37.5%) and 4/8 (50%) respectively as shown in Table 1.

The overall of pregnancy rates were 9/16 (56.25%) in $PGF_{2\alpha}$ group, 10 /16 (62.5%) in ovsynch group and 7/16 (43.75%) in control group with a high significant ($P< 0.001$) increase in ovsynch group rather than control group and $PGF_{2\alpha}$ group as shown in Table 1.

Table 1: Pregnancy rates in different groups (%)

Animal Group	Primiparous	Multiparous	tal
GroupI ($PGF_{2\alpha}$)	50.0 (4\8)^{a}	62.5 (5\8)^{b}	56.25 (9\16)^{B}
Group II (Ovsynch)	50.0 (4\8)^{a}	75.0 (6\8)^{a}	62.5 (10\16)^{A}
Group III (Control)	37.5 (3\8)^{b}	50.0 (4\8)^{c}	43.75 (7\16)^{C}

Total rates with different capital letters superscript in the last column differs significantly (P ≤0.001).

Pregnancy rates with different small letters superscripts in the same column differs significantly (P ≤0.001).

Progesterone concentration:

As presented in Table (2) serum concentration of progesterone were not different for primiparous buffaloes in Groups I , II and III just before treatment (0.686 ± 0.115, 0.755 ± 0.076 and 0.676 ± 0.045 ng/ml respectively,) while, in multiparous buffaloes in Groups I, II and III were (0.927 ± 0.157 ,0.722 ± 0.058 and 0.767 ± 0.085 ng/ml, respectively).

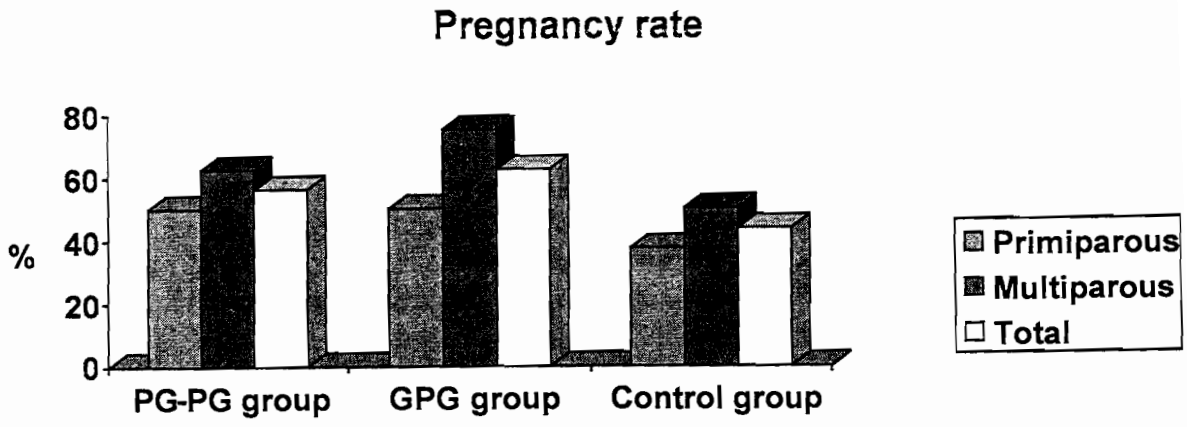
Regarding progesterone level after 25 days post treatment, it is found that the group II has a higher significant increase (P ≤001) (4.886 ± 0.333 ng/ml) for primiparous and (5.083 ± 0.100 ng/ml) for multiparous buffaloes; than group I (4.684 ± 0.126, 4.734 ± 0.230ng/ml); and group III (4.837± 0.353, 4.239 ± .124 ng/ml) primiparous and multiparous pregnant animals, respectively.

Table 2: Serum progesterone profile for different groups (ng\ml) (Mean ± SEM)

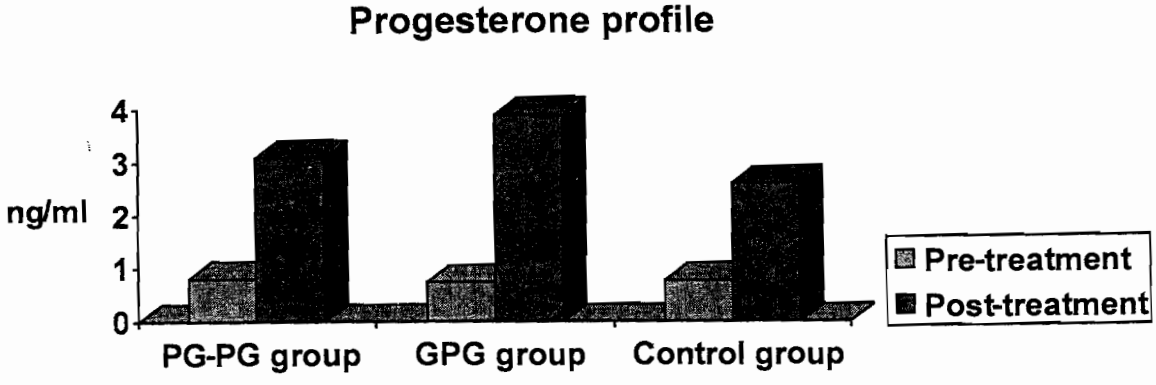
Animal groups	Pretreatment (on day 0)		Post insemination (on day 25)			
	Primiparous	Multiparous	Non- Pregnant		Pregnant	
			Primiparous	Multiparous	Primiparous	Multiparous
G I (PG – PG)	N=8 0.686^{b} ± 0.115	N=8 0.927^{a} ± 0.157	N=4 1.118^{Ba} ± 0.076	N=3 0.865^{Cb} ± 0.288	N=4 4.684^{Bb} ± 0.126	N=5 4.734^{Ba} ± 0.230
G II (Ovsynch)	N=8 0.755^{b} ± 0.076	N=8 0.722^{a} ± 0.058	N=4 2.473^{Aa} ± 0.448	N=2 1.139^{Bb} ± 0.044	N=4 4.886^{Aa} ± 0.333	N=6 5.083^{Aa} ± 0.100
G III (Control)	N=8 0.676^{b} ± 0.045	N=8 0.767^{a} ± 0.085	N=5 0.903^{Cb} ± 0.042	N=4 1.385^{Aa} ± 0.096	N=3 4.837^{Aa} ± 0.353	N=4 4.239^{Cb} ± 0.124

Progesterone levels (on day 0) with different small alphabetical superscripts in the same column differs significantly (P ≤0.001).

Progesterone levels with different capital alphabetical superscript in the same column differ significantly at least at p ≤ 0.001. while, progesterone level (either in pregnant or in the non- pregnant group in the same raw) with different small alphabetical superscript differ significantly at least at (p ≤0.01).



ıe Pregnancy rates (%) in different groups.



erum progesterone profile (ng/ml) in different groups.

As shown in figure 1 pregnancy rate was higher in group II than other groups, while Figure 2 showed progesterone profile was higher comparatively in group II than other groups.

DISCUSSION

The main purpose of this study was to evaluate the effect of $PGF_{2\alpha}$ as a way of synchronization of estrus and the Ovsynch program for ovulation synchronization with pregnancy rates in Egyptian buffaloes. $PGF_{2\alpha}$ group resulted in 56.25% pregnancy rate in lactating buffaloes these results corresponded to the results in cows by Lucy *et al.*, (1986); Stevenson *et al.* (1987) and Archbald *et al.* (1992) that possibly due to the variability in time from AI to ovulation. This variability in time from injection of $PGF_{2\alpha}$ to estrus and subsequent ovulation may be directly related to the number and size of the ovulatory follicles at the time of $PGF_{2\alpha}$ injection. In contrast to the Ovsynch protocol that showed pregnancy rate 62.5% which is considered high significance ($P< 0.001$) rather than other groups ($PGF_{2\alpha}$ group and control group),this result agreed with those applied on Brazilian buffaloes by Roy *et al.* (1996); Burke *et al.* (1996); Momcilovic *et al.* (1998); Berber *et al.* (2002); Bartolomeu *et al.* (2002) and Paul and prakash (2005). A high percentage of pregnancy rate in lactating buffaloes (75%) at a random stage of the estrus cycle ovulated a follicle after first injection of GnRH specially in multiparous rather than primiparous that was in accordance with that results in cows revealed by Silcox *et al.* (1993). The difference between pregnancy rates of primiparous (50%, 50%, 37.5%) and multiparous (62.5%, 75%, 50%) in the different groups respectively agreed with Pietro *et al.* (2003) and demonstrated that the parity is a decisive factor in the efficiency of the protocols. Also, they reported that primiparous spend energy for the continuity of the corporal growth and multiparous converge that energy for the reproductive processes.

In regarding to plasma progesterone concentrations of pregnant buffaloes in groupI and II tended to be higher in multiparous buffaloes 4.734±0.230 ng/ml and 5.083±0.100 ng/ml than in multiparous of group 3 (4.239 ±0.124ng/ml while they are not significant to the corresponding levels of the primiparous buffaloes 4.684 ± 0.126 ng/ml and 4.886 ±0.333 ng/ml in group I and II respectively that agreed with Mee, *et al.* (1993). This may be attributed to high level of progesterone concentrations of GPG buffaloes were probably due to the stimulatory effects of GnRH, or to prolonged effects of GnRH on the proportion of large luteal cells in the developing corpus luteum during pregnancy. So, estrus expression is limited with $PGF_{2\alpha}$ than GPG protocol based on fixed-time insemination protocols in lactating buffaloes and necessitates

minimal amounts of estrus detection in order to reach maximal pregnancyrates.

REFERENCES

Archbald, L.F.; Tran, T.; Massey, R. and Klapstein, E. (1992): nception rates in dairy cow after timed-insemination and simultaneous treatment with gonadotrophin-releasing hormone and/or prostaglandin F2α Theriogenology. 37: 723-226.

Bartolomeu, C.C.; Del Rei, A.J.M.; Madureira, E.H.; Souza, A.J.; Silva, A.O. and Baruselli, P.S. (2002): Timed insemination using synchronization of ovulation in buffaloes using CIDR-B, CRESTAR and Ovsynch. Anim. Breed Abstr. 70: 332 -235.

Baruselli, P.S.; Madureira, E.H.; Barnabe, V.H.; Barnabe, R.C.; Berber, R.C.A. and Amaral, R. (2000): Timed insemination using synchronization of ovulation in buffalo. 14th International Congress of Animal Reproduction, Barazelu, V. 14, P. 39 -43.

Baruselli, P.S.; Amaral, R.; Barufi, F.B.; Valentim, R. and Marques, M.O. (2001): Lecirelin and Buserelin (gonadotrophin-releasing hormone agonists) are equally effective for fixed timed insemination in buffalo. Braz. J. Vet. Res. Anim. Sci. Vol.38 No.3 P.215-218, Sao. Paulo.

Baruselli, P.S.; Madureira, E.H.; Banarbe, V.H.; Barnabe, R.C. and Berber, R.C.A. (2003): Evaluation of synchronization of ovulation for fixed timed insemination in buffalo (Bubalus bubalis). Braz. J. Vet. Res. Anim. Sci. Vol.40 No.6 P. 433-437, Sao. Paulo.

Berber, R.C.; de 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.

Britt, J.S. and Gaska, J. (1998): Comparison of two estrus synchronization programs in a large, confinement-housed dairy herd. JAVMA.212:210-212.

Burke, J.M.; De la Sota, R.L.; Risco, C.A.; Staples, C.R.; Schmitt, E.J-P. and Thatcher, W.W. (1996): Evaluation of timed insemination using a gonadotrophin-releasing hormone agonist in lactating dairy cows. J. Dairy Sci. 79: 1385-1393.

King, M.E.; Kirakofe, S.D.; Stevenson, J.S. and Schalles, R.R. (1982): Effect of stage of the estrous cycle on interval to estrus after $PGF_{2\alpha}$ in beef cattle. Theriogenology. 18: 191.

Larson, L.L. and Ball, P.J.H. (1992): Regulation of estrous cycles in dairy cattle: a review. Theriogenology. 38: 255.

Lucy, M.C.; Stevenson, J.S. and Call. E.P (1986): Controlling first service and calving interval by prostaglandin F2α gonadotrophin-releasing hormone and timed insemination. J. Dairy Sci. 69: 2186.

Mee, O.M.; Stevenson, J.S.; Alexander, B.M. and Sasser, R.G. (1993): Administration of GnRH at estrus influences pregnancy rates, serum concentration of LH, FSH, estradiol-17B, pregnancy–specific protein b, and progesterone, proportion of luteal cell types, and in vitro production of progesterone in dairy cows. J. Anim. Sci.71,185-198.

Momcilovic, D.; Archbald, L.F.; Walters, A.; Tran, T.; Kelbert, D. and Risco, C. (1998): Reproductive performance of lactating dairy cows treated with gonadotrophin-releasing hormone (GnRH) and\or prostaglandin F2α ($PGF_{2\alpha}$) for synchronization of estrus and ovulation. Theriogenology, 50: 1131-1139.

Momont, H.W. and Seguin, B.E. (1983): Treatment of unobserved estrus in lactating dairy cows with prostaglandin F2α products. Page 28 in The Compendium on Continuing Education for the Practicing Veterinarian on Reproductive Management in Food Animals. Univ. Minnesota, St. Paul.

Nebel, R.L. and Jobst, S.M. (1998): Evaluation of systematic breeding program for lactating dairy cows: a review. J. Dairy Sci. 81: 1169- 1174.

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.

Pietro S.B.; Ed Hoffman M.; Valquiria H.B.; Renato C.B. and Rodolfo C.B. (2003): Evaluation of synchronization of ovulation for fixed timed insemination in buffalo (Bubalus bubalis). Brazilin J. Vet. Res. Anim. Sci. 40: 6: Sao Paulo. Aproved para puplication: 17/09/2003.

Pursley, J.R.; Kosorok, M.R. and Wiltbank, M.C. (1997a): Reproductive management of lactating dairy cows using synchronization of ovulation. J. Dairy Sci .80: 301 -306.

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.

Pursley, J.R.; Wiltbank, M.C.; Stevenson, J.S.; Ottobre, J.S.; Garverick, H.A. and Anderson, L.L. (1997b): Pregnancy rates per artificial insemination for cows and heifers inseminated at a synchronized ovulation or synchronized estrus. J. Dairy Sci. 80: 295-300.

Roy, G.L. and Twargiramungu, H.A. Fixed –time AI program using the GnRH- $PGF_{2\alpha}$ –GnRH method for beef females. J. Anim. Sci. 74 (suppl.): 222 (Abstr).

Silcox, R.W., Powell, K.L. and Kiser, T.E. (1993): Ability of dominant follicles (DF) to respond to exogenous GnRH administration is depended on their stage of development. J. Anim. Sci. 71 (Suppl 1): 513 Abstr.

Snedecor, G.W. and Cochran, W.G. (1980): Statistical methods 7th ed. J.B.H publishing Comp. Oxford.

Stevenson, J.S.; Kobayashi, Y. and Thompson, K.E. (1999): Reproductive performance of dairy cows in various programmed breeding systems including Ovsynch and combination of gonadotrophin-releasing hormone and prostaglandin F2α . J. Dairy Sci. 82: 506- 515.

Stevenson, J.S.; Lucy, M.C. and Call, E.P. (1987): Failure of timed inseminations and associated luteal function in dairy cattle after two injections of prostaglandin F2α. Theriogenology. 28: 937.

Stevenson, J.S.; Mee, M.O. and Stewart, R.E. (1989): Conception rates and calving intervals after prostaglandin F2α or rebreeding progesterone in dairy cows. J. Dairy Sci. 72: 208.

Stevenson, J.S.; Smith, J.F. and Hawkins, D.E. (2000): Reproductive outcomes of dairy heifers treated with combination of prostaglandin F2α, norgestomet, and gonadotrophin-releasing hormone. J. Dairy Sci. 83: 1- 8.