Alex. J. Agric. Res. 54 (2) 63-69, 2009

EFFECTIVENESS OF GnRH/PGF$_{2\alpha}$-BASED PROTOCOLS ON REPRODUCTIVE EFFICIENCY OF HOLSTEIN HEIFERS

Samir Z. El-Zarkouny

Received on: 24/6/2009 ***Accepted: 30/6/2009***

ABSTRACT

The objective of this study was to evaluate the effectiveness of GnRH/PGF-based protocols on reproductive outcomes of Holstein heifers. Heifers were assigned randomly to either controls (n = 14) or those treated (n = 50) with GnRH (d - 7) and PGF2α (d 0). Heifers that displayed estrus were inseminated (Select Synch, n = 34), and those that did not display estrus up to 48 h after PGF$_{2\alpha}$ injection received a second GnRH injection and time-inseminated (Ovsynch, n = 16). Conception rates were 40, 55.9 and 31.3% and overall mean pregnancy rates ($P < 0.05$) were 64.3, 97.0, and 87.5% for control, Select Synch, and Ovsynch heifers, respectively. Ovsynch heifers had 1.8 ± 0.5 and 3.2 ± 0.6 ng/ml of serum P4 levels compared to 3.3 ± 0.5 and 3.8 ± 0.6 ng/ml for the Select Synch heifers at the times of first GnRH and PGF$_{2\alpha}$ injections, respectively. Conception rates were 55.6 and 58.3% when P4 concentrations were high compared to 50 and 25% when P4 concentrations were low at the times of first GnRH and PGF$_{2\alpha}$ injections, respectively. Dairy heifers synchronized with GnRH and PGF$_{2\alpha}$ should be inseminated at detected estrus, and heifers not displaying estrus up to 48 h after PGF$_{2\alpha}$ injection should be given a second GnRH and time-inseminated 16-20 h later.

Key words: heifers, conception rates, progesterone, Ovsynch, Select Synch.

INTRODUCTION

Replacement heifers are the future of the dairy farm (Tenhagen et al., 2005). Reproductive management of dairy heifers has always been a challenge. In recent years, a combination of GnRH and PGF$_{2\alpha}$ has been used to increase the reproductive efficiency in lactating dairy cows and heifers (Yaniz et al., 2004). Ovulation-synchronization (Ovsynch) protocol was the first protocol designed to synchronize ovulation in dairy cows that allows for timed artificial insemination (TAI) with acceptable pregnancy rates (Pursley et al., 1995). However, heifers did not respond favorably as pregnancy rates were 20 to 40% lower than in heifers allowed to display estrus before insemination (Schmidit et al., 1996, Pursley et al., 1997). Fewer studies by Peckelhof et al. (2000) and Tenhagen et al. (2005) assessed the reproductive outcomes of estrus synchronization in dairy heifers using GnRH and PGF$_{2\alpha}$ 7 days apart followed by AI on observed estrus. Some studies have assessed the effectiveness of synchronizing ovulations using GnRH and PGF$_{2\alpha}$ followed by TAI in dairy heifers but results were inconsistent due to low numbers of heifers (Moreira et al., 2000) or to an unknown synchronization response (Pursley et al., 1997). Decreased pregnancy rates in nulliparous heifers on the Ovsynch protocol could be due to the number of follicular waves (Pursley et al., 1997) or to inconsistent follicular wave emergence (Nebel and Jobst, 1998). Ovulation of the ovulatory follicle occurred within 24-48 h after the second GnRH injection in the Ovsynch protocol in dairy cows (Taponen et al., 1999; Vasconcelos et al., 1999; Tenhagen et al., 2003, 2005). Greater proportion (47.1%) of ovulations were detected in dairy heifers within 18-24 h after the second GnRH injection in the Ovsynch protocol compared to 10% in Multiparous cows (Demiral et al., 2006). Therefore, late application of TAI and/or aged oocytes could be the reason for decreased pregnancy rates in the Ovsynch protocol in dairy heifers (Demiral et al., 2006).

The objective of the current study was to evaluate the effectiveness of estrus synchronization protocol that allows for AI based on estrus observation in Holstein heifers treated with GnRH before PGF$_{2\alpha}$-induced luteolysis and estrus (GnRH + PGF$_{2\alpha}$; Select Synch) compared to the Ovsynch protocol plus timed AI.

MATERIALS AND METHODS

The current study was conducted in a private dairy farm (Alalameia for Animal Production and Land Reclamation) located 90 km south of Alexandria city on the desert road between Alexandria and Cairo. All heifers were born in that dairy farm by Holstein cows inseminated with frozen semen imported from USA.

Heifers' management: All heifers were housed in open barns in groups of 10 animals based on their age and body weight. They were raised on heifers' feed consisting of silage and concentrate. Heifers were allotted randomly to treatment protocols based on their age and body weight requirements at the start of treatment. The minimum inclusion age was 13-month and 350 kg body weight.

Department of Animal Production, Faculty of Agriculture, Alexandria University, Alexandria, Egypt

Treatment protocols: The experimental design is presented in Figure 1. The first group of heifers (n = 14) did not receive any treatment and served as control. Heifers in the control group were observed for signs of behavioral estrus twice daily throughout the entire treatment period and were artificially-inseminated (AI) according to the AM/PM rule. Estrus was synchronized in the rest of heifers (n = 50) with gonadotropin releasing hormone (GnRH) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). Synchronized heifers were injected with 20 µg of GnRH1 (Busereline acetate-Receptal®) on day (-7), and 0.150 mg $PGF_{2\alpha}$ (Cloprestenol - Estrumate®) on d (0). All heifers were observed for signs of behavioral estrus starting 24 h after first GnRH injection and continued up to 48 h after $PGF_{2\alpha}$ injection. Heifers that displayed estrus were inseminated 8-16 h later (Select Synch; n = 34) and received no further injections. Heifers that did not display estrus within the 48 h-timeframe after $PGF_{2\alpha}$ injection were injected with a second injection of GnRH (Ovsynch, n = 14) and were time-inseminated 16 -20 h after GnRH injection.

Blood collection and Enzyme immunoassays: Blood samples were collected via tail vein puncture before hormonal treatments on d -7 (first GnRH injection), d 0 ($PGF_{2\alpha}$ injection), and d + 2 (second GnRH injection) in the Select Synch and Ovsynch heifers, and on the corresponding days in the control heifers. Blood samples were centrifuged at 3000 rpm for 20 min and sera were harvested and stored at -20°C for later analysis of progesterone hormone. Progesterone (P4) was measured in serum samples using Enzyme Immunoassay kits. Intra and inter assay coefficients of variation for P4 were 4.4 and 4.3%, respectively. Conception rates to first service were determined by ultrasound scanning of uterine contents using an ultrasound system (Dynamic Imaging Concept MLV, Livingston, Scotland) equipped with a dual 5.0-7.5 MHz transrectal transducer 35.0 ± 5 days after AI. Numbers of services per conception were determined for control, Ovsynch and Select Synch heifers.

Statistical analyses: Rates of AI submission based on visual detection of estrus, conception rate (number of pregnant heifers after synchronized insemination divided by the number of heifers inseminated), overall pregnancy rate (%) (number of pregnant heifers after the end of AI period), and number of services per conception to first AI were calculated. Interval from $PGF_{2\alpha}$ injection (d 0) to first standing of estrus was calculated. All the preceding variables were analyzed by GLM procedure in SAS (1999). Categories of P4 (high and low) serum, time of injections (GnRH 1, $PGF_{2\alpha}$, and GnRH 2), and treatments were used as main sources of variation on conception rates to first AI in a separate model (SAS).

RESULTS

Data for conception rates (%) to first service (CR1), number of services per conception (NSC), and overall mean conception rates (CR, %) are presented in Table 1. Heifers treated with the Ovsynch protocol had 31.3% conception rate to first service compared to 40.0% and 55.9% for the controls and Select Synch heifers, respectively. There were no significant differences in the number of services per conception (2.3, 1.8, and 1.7) among the control, Ovsynch, and Select Synch heifers, respectively. Overall mean conception rates for Select Synch (97.0%) and Ovsynch (87.5%) heifers were greater ($P<0.05$) than controls (64.3%).

Average serum concentrations of P4 (ng/ml) at the times of first GnRH, $PGF_{2\alpha}$ and second GnRH injections in the control, Ovsynch, and Select Synch heifers are presented in Table 2. At the time of first GnRH injection, no differences were detected in the average serum concentrations of P4 in the three groups of heifers. However, first GnRH injection resulted in decreased ($P < 0.05$) serum P4 concentrations at the time of $PGF_{2\alpha}$ injection (3.2 and 3.8 ng/ml in the Ovsynch and Select Synch Heifers, respectively) compared to controls (6.1 ng/ml). Heifers treated with $PGF_{2\alpha}$ (Ovsynch and Select Synch) had lower ($P < 0.05$) serum P4 concentrations (1.7 ng/ml) at the time of second GnRH injection compared to control heifers (5.7 ng/ml).

Percentages of heifers with decreased serum progesterone concentration at the time of second GnRH injection after $PGF_{2\alpha}$ injection were almost similar (58.3% and 54.6% in the Ovsynch and Select Synch protocols, respectively). Only 25% of control heifers had spontaneous luteal regression and decreased serum P4 concentrations at the time of second GnRH injection to the Ovsynch and Select Synch heifers.

Further relationships between serum P4 and conception rates are summarized in Figure 2. Conception rate in heifers with luteal activity ($P4 \geq 1.0$ ng/ml) before $PGF_{2\alpha}$ injection averaged 55.6% (45.5 and 71.4% in the Ovsynch and Select Synch-treated heifers, respectively). However, conception rate in heifers that had low (< 1.0 ng/ml) serum P4 concentrations before $PGF_{2\alpha}$ injection was 50% (100 and 33.3% in the Ovsynch and Select Synch-treated heifers, respectively). Conception rate in heifers with luteal activity ($P4 > 1.0$ ng/ml) at the time of $PGF_{2\alpha}$ injection was 58.3% (57.1 and 60% in the Ovsynch and Select Synch-treated heifers, respectively), and in heifers with low (< 1.0 ng/ml) serum P4 concentrations was 25% (33.3 and 0.0% in the Ovsynch and Select Synch-treated heifers, respectively).

Of all the treated heifers (n = 50), 34 (68%) were detected in estrus and therefore were inseminated as (Select Synch) heifers, and as a result fewer ($P < 0.01$) heifers 16 (32%) were not detected in estrus up to 48 h after $PGF_{2\alpha}$ injection and were given the second GnRH injection and were time-inseminated (Ovsynch).

Proportional distribution of estrus behavior in the Select Synch heifers starting from the date of first GnRH injection up to 48 h after $PGF_{2\alpha}$ injection is shown in Figure 3. After $PGF_{2\alpha}$ injection, 29.55 and 38.64% of heifers treated with first GnRH injection and $PGF_{2\alpha}$ displayed estrus within 24 and 48 h, respectively. Four more heifers were detected in estrus on days - 6, - 2, - 1 and 0 of $PGF_{2\alpha}$ injection (one heifer each day).

DISCUSSION

Pregnancy rate per AI was reported to be lower for heifers treated with Ovsynch than for control group that were synchronized with $PGF_{2\alpha}$ (Pursley et al., 1997). This could be explained by a lower ovulatory response to first GnRH injection and to some other differences in follicular dynamics of heifers. Some investigators (Taponen et al., 1999; De Araujo Berber et al., 2002) also indicated that the presence of CL or elevated progesterone concentrations at the beginning of the ovulation-synchronization protocol could be a determining factor for the success of such protocol.

In the present study, heifers that displayed estrus after $PGF_{2\alpha}$ injection (Select Synch) had elevated (3.3 ± 0.5 ng/ml) P4 concentration at the time of first GnRH injection compared to (1.8 ± 0.5 ng/ml) in heifers that did not display estrus (Ovsynch). It has been demonstrated that the success of the Ovsynch program is influenced by the stage of estrus cycle when the first GnRH dose was administered (Keister et al., 1999; Vasconcelos et al., 1999; Moreira et al., 2000). Pregnancy rates were increased when dairy cows were presynchronized with two $PGF_{2\alpha}$ injections (Moreira et al., 2000) or one $PGF_{2\alpha}$ injection (Cartmill et al., 2001) before the start of the Ovsynch protocol. Presynchronization of the estrous cycle put the cows in a proper stage of the estrous cycle (early or late diestrus) before the start of the Ovsynch protocol.

The overall mean conception rates in this study are in accordance with previous reports showing that pregnancy rates are higher in heifers with high P4 concentration at the time of $PGF_{2\alpha}$ injection compared to heifers with low serum P4 concentration. Furthermore, decreased overall mean pregnancy rates in heifers with low serum P4 concentration at the time of $PGF_{2\alpha}$ injection is compatible with previous reports by Pursley et al. (1997) indicating that the presence of a functional CL or high concentration of P4 at the time of $PGF_{2\alpha}$ injection is a critical factor for success of ovulation-synchronization protocols.

Burke et al. (1996) recorded higher conception rates in cows undergoing AI at detected estrus (Ovsynch without the second GnRH injection) compared to cows time-inseminated (Ovsynch). Timed AI following the Ovsynch protocol was strongly advocated by several investigators (Burke et al., 1996; Momcilovic et al., 1998; Yamada et al., 1999) as an effective tool in improving reproductive management in dairy cows because it eliminates estrus detection. De Jarnette et al. (2001) found that 20% of Ovsynch-treated cows displayed premature estrus (48 h after $PGF_{2\alpha}$) and concluded that estrus detection during this period improved conception rates. In the current study, injection of GnRH and $PGF_{2\alpha}$ caused more heifers (34/50) to display estrus behavior and as a result they were inseminated (Select Synch), and (16/50) of heifers did not display estrus up to 48 h after $PGF_{2\alpha}$ injection and were injected with second GnRH and were time-inseminated (Ovsynch).

Some scientists reported that ovulation in cows occurs within 24–48 h after the second GnRH injection in Ovsynch procedure (Taponen et al., 1999; Vasconcelos et al., 1999; Tenhagen et al., 2003, 2005). Furthermore, the highest proportions were obtained between 24 to 32 h (Pursley et al., 1995; Demiral et al., 2006). Demiral et al. (2006) detected 47.1% of ovulations in dairy heifers in the 16-20 h time interval (second GnRH injection - AI) and concluded that late application of timed AI in heifers treated with the Ovsynch protocol would result in aging of the oocytes and reduce pregnancy rates in heifers. Estrus detection 48 h after $PGF_{2\alpha}$ injection is considered as an acceptable practice to maximize pregnancy rates before the second GnRH injection during the Ovsynch protocol in dairy heifers (Stevenson et al., 2000).

Replacement dairy heifers synchronized with GnRH and $PGF_{2\alpha}$ should be monitored closely for signs of behavioral estrus and inseminated at detected estrus, and heifers not displaying estrus up to 48 h after $PGF_{2\alpha}$ injection should be given a second GnRH and time-inseminated 16 – 20 h later.

Table (1): Reproductive traits conception rate (CR1), number of services per conception (NSC) of dairy heifers treated with the Ovsynch and Select Synch protocols and controls.

Treatment	CR1 (%)	Overall mean CR (%)	NSC
Control	40.0 (n=10)	64.3^b (n=14)	2.3 ± 0.3 (n=10)
Ovsynch	31.3 (n=16)	87.5^a (n=16)	1.8 ± 0.3 (n=16)
Select Synch	55.9 (n=34)	97.0^a (n=34)	1.7 ± 0.2 (n=34)

CR1: Conception rate to first service
Values within columns with different superscripts differ (P<0.05)

Table (2): Average serum concentrations of progesterone (ng/ml) and luteolysis (%) in dairy heifers receiving the Ovsynch and Select Synch protocols and controls.

	Control	Ovsynch	Select Synch
GnRH1*	3.6 ± 0.8	1.8 ± 0.5	3.3 ± 0.5
PGF$_{2\alpha}$*	6.1 ± 1.0^a	3.2 ± 0.6^b	3.8 ± 0.6^b
GnRH2*	5.7 ± 0.7^a	1.7 ± 0.4^b	1.7 ± 0.4^b
Luteolysis (%)	25.0	58.3	54.6

GnRH1: First GnRH injection
GnRH2: Second GnRH injection
* P4 (ng/ml)
Values within rows with different superscripts differ (P<0.05).

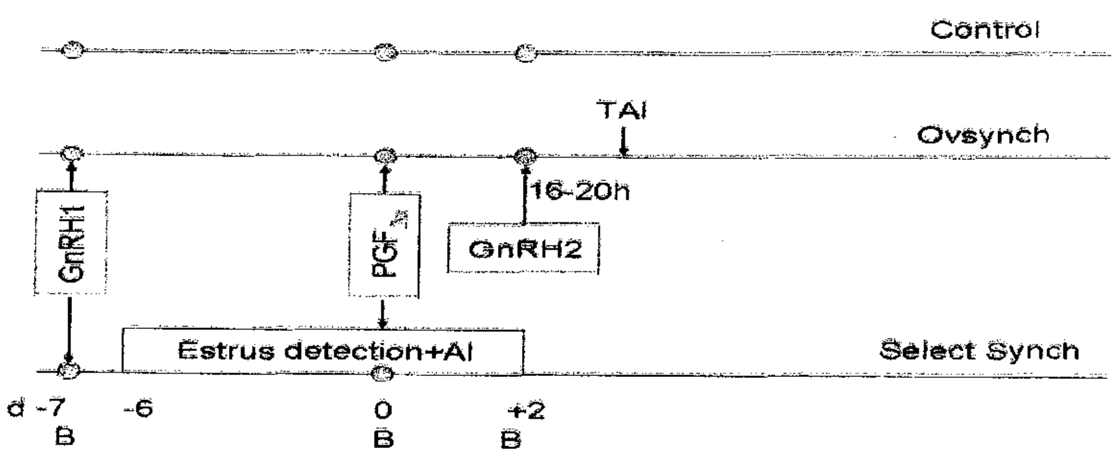

Fig. (1): Experimental design for dairy heifers treated with control, Ovsynch and Select Synch protocols. Blood (B) samples were collected from all animals in days -7, 0, and + 2.

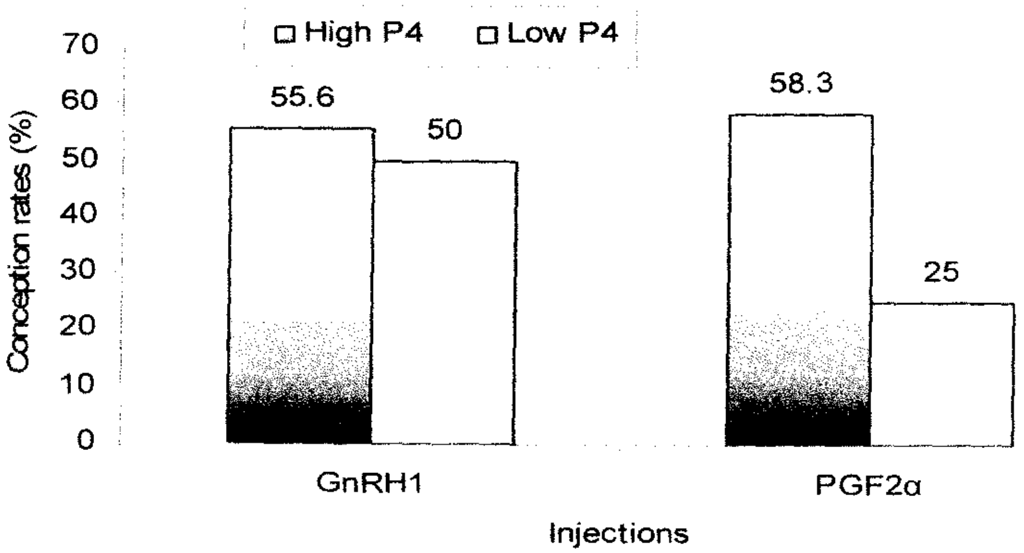

Fig. (2): Conception rates in Holstein heifers when P4 concentrations were high (≥ 1 ng/ml) or low (< 1 ng/ml) at the times of first GnRH and $PGF_{2\alpha}$ injections.

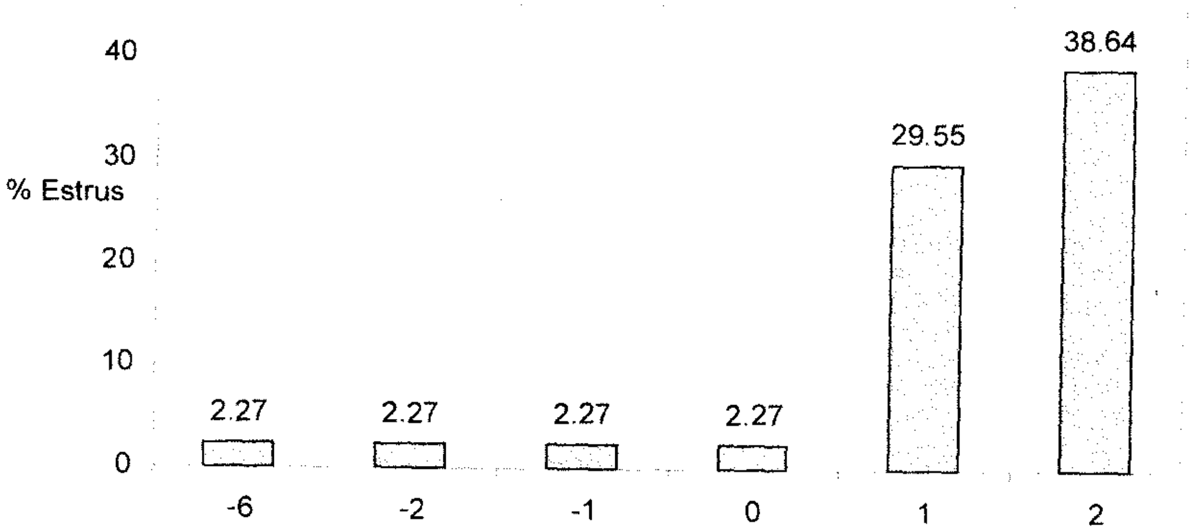

Fig. (3): Percentage of distribution of estrus before and after $PGF_{2\alpha}$ injection in Holstein heifers treated with GnRH and $PGF_{2\alpha}$ (Select Synch).

REFERENCES

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

Cartmill, J.A., S.Z. El-Zarkouny, B.A. Hensley, G.C. Lamb and J.S. Stevenson. (2001). Stage of cycle, incidence and timing of ovulation, and pregnancy rates in dairy cattle after three timed breeding protocols. J. Dairy Sci. 84: 1051-1059.

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

De Jarnette J.M., R.R. Salverson and C.E. Marshall (2001). Incidence of premature estrus in lactating dairy cows and conception rates to standing estrus or fixed-time inseminations after synchronization using GnRH and PGF2α. Anim. Reprod. Sci. 67: 27–35.

Demiral, O., M. Űn, M. Abay, T. Bekyurek and A. Őztűrk (2006). The effectiveness of Cosynch protocol in dairy heifers and Multiparous cows. Turk. J. Vet. Anim. Sci. 30: 213-217.

Keister, Z.O., S.K. DeNise, D.V. Armstrong, R.L. Ax, and M.D. Brown (1999). Pregnancy outcomes in two commercial dairy herds following hormonal scheduling programs. Theriogenology. 51: 1587-1596.

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

Moreira, F., R.L. de la Sota, T. Diaz and W.W. Thatcher (2000). Effect of day of the estrous cycle at the initiation of a timed artificial insemination protocol on reproductive responses in dairy heifers. J. Anim. Sci. 78: 1568-1576.

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

Peckelhoff, H., S. Kuchenbuch, S. Kűne, A. Biedermann, and W. Heuwieser (2000). Fruchtbarkeitsmanagement bei Fărsen durch Ovulations synchronisation. Tierăztl Prax. 28: 88-92.

Pursley, J.R., M.O. Mee, and M.C. Wiltbank (1995). Synchronization of ovulation in dairy cows using PGF2α and GnRH. Theriogenology 44: 915-923.

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

SAS Institute (1999). SAS/STAT User's Guide. Version 8.2. SAS Institute, Inc., Cary, NC.

Schmidt, E.J.P., T. Diaz, M. Drost and W.W. Thatcher (1996). Use of a gonadotropin-releasing hormone agonist or human chorionic gonadotropin for timed insemination in cattle. J. Anim. Sci. 74: 1084-1091.

Stevenson, J.S., J.F. Smith, and D.E. Hawkins. 2000. Reproductive outcomes for dairy heifers treated with combinations of prostaglandin F2α, Norgestomet, and gonadotropin-releasing hormone. J Dairy Sci. 83: 2008-2015.

Taponen, J., T. Katila and H. Rodriguez-Martinez (1999). Induction of ovulation with gonadotropin-releasing hormone during proestrus in cattle: influence on subsequent follicular growth and luteal function. Anim. Reprod. Sci. 55: 91-105.

Tenhagen, B.A., S. Kuchenbuch and W. Heuwieser (2005). Timing of ovulation and fertility of heifers after synchronization of oestrus with GnRH and prostaglandin F2α. Reprod. Domest. Anim. 40: 62-67.

Tenhagen, B.A., M. Wittke, C. Vogel, M. Drillich and W. Heuwieser (2003). Timing of ovulation and conception rate in primiparous and multiparous cows after synchronization of ovulation with GnRH and PGF2α. Reprod. Domest. Anim. 38: 451-454.

Vasconcelos, J.L.M., R.W. Silcox, G.J.M. Rosa, J.R. Pursley, and M.C. Wiltbank (1999). Synchronization rate, size of the ovulatory follicle, and pregnancy rate after synchronization of ovulation beginning on different days of the estrus cycle in lactating dairy cows. Theriogenology 52: 1067-1078.

Yamada, K., T. Nakao and N. Mihara. (1999). Synchronization of ovulation and fixed-time insemination for improvement of conception rate in dairy herds with poor estrus detection efficiency. J. Reprod. Dev. 45: 51-55.

Yaniz, J.L., K. Murugavel, and F. Lopez-Gatius (2004). Recent development in oestrous synchronization of postpartum dairy cows with and without ovarian disorders. Reprod. Domestic. Anim. 39: 86-93.

الملخص العربي

كفاءة إستخدام الجونادوتروبينات والبروستاجلاندين $PGF_{2\alpha}$ على الكفاءة التناسلية لعجلات الهولستين

سمير زكي الزرقوني

قسم الإنتاج الحيواني – كلية الزراعة – جامعة الإسكندرية

الهدف من الدراسة هو تقييم تأثير إستخدام بروتوكول $GnRH/PGF_{2\alpha}$ على التناسل فى عجلات الهولشتين. تم تقسيم العجلات عشوائياً إلى مجموعتين: المجموعة الأولى الضابطة وعددهم ١٤ عجلة والمجموعة الثانية (٥٠ عجلة) عوملت بـ GnRH ثم بعد سبعة ايام بـ $PGF_{2\alpha}$. الحيونات التى ظهرت عليها علامات الشياع (٣٤ عجلة ، Select Synch) تم تلقيحها بينما العجلات التى لم تظهر عليها علامات الشياع حتى ٤٨ ساعة من حقن الـ $PGF_{2\alpha}$ تم حقنها مرة اخرى بـ GnRH (١٦ عجلة، Ovsynch). أظهرت النتائج أن معدلات الحمل من اول تلقيحه كانت ٤٠ و ٥٥,٩٢ و ٣١,٣ % بينما المتوسط العام لمعدلات الحمل كانت ٦٤,٣ و ٩٧ و ٨٧,٥ % فى حالة المجموعة الضابطة و Select Synch و Ovsynch على الترتيب. تركيز البروجسترون فى عجلات الـ Ovsynch كان ١,٨ و ٣,٢ نانوجرام / مل مقارنة بـ ٣,٣ و ٣,٨ نانوجرام / مل فى حالة عجلات Select Synch فى أوقات أول حقنة GnRH وعند حقن الـ $PGF_{2\alpha}$ على الترتيب. معدلات الحمل كانت ٥٥,٦ و ٥٨,٣ % عندما كان تركيز البروجسترون عالى مقارنة بـ ٥٠ و ٢٥ % عندما كان تركيز البروجسترون منخفض فى وقت الحقنة الأولى من GnRH وعند حقن الـ $PGF_{2\alpha}$ على الترتيب .

أوضحت الدراسة أنه لزيادة كفاءة استخدام برنامج تنظيم الشياع (Ovsynch) المعتمد على الـ GnRH و $PGF_{2\alpha}$ يجب أن تلقح العجلات عند إكتشاف الشباع بعد حقن الـ $PGF_{2\alpha}$ بينما العجلات التى لم تظهر عليها علامات الشياع يجب أن تحقن مرة أخرى بالـ GnRH وتلقح بعد ١٦– ٢٠ ساعة.

الكلمات الدالة: عجلات ماشية اللبن – معدلات الحمل – البروجستيرون – Ovsynch- Select Synch.