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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 10 (20): 3557-3563, 2007
ISSN 1028-8880

Comparison Between Different Protocols of Synchronization and Their Efficiency on Pregnancy Rate of Dairy Cattle

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Abstract: In order to determine different protocols of synchronization and their efficiency on pregnancy rate after fixed-timed AI (TAI), 120 dairy Holstein cows (n = 120) were assigned randomly to six groups: 1) two injections of Prostaglandin $F_{2\alpha}$ ($PG_{f2\alpha}$) with 12 days apart as a control group, 2) two injections of Gonadotropin Releasing hormone (GnRH) with 9 days apart and an injection of $PG_{f2\alpha}$ at day 7, 3) injection of GnRH and $PG_{f2\alpha}$ with 7 days apart, then single injection of Estradiol Benzoate (EB) after 48 h, 4) injection of progesterone (P4) in conjugation with EB then after 7 days $PG_{f2\alpha}$ injection and after 48 h an injection of GnRH were done, 5) as group 4 but EB was used instead of GnRH, 6)injections of $PG_{f2\alpha}$ and EB conjugated with Human Chronic Gonadotrophin (hCG) with 12 h apart. Animals in group 1 (control), groups 2-5 and group 6 were inseminated after 72, 20 and 36 h, respectively. Serum P4 concentration of group 4 (4.43± 1.50 ng mL^{-1}) was higher than control group (2.34±1.36 ng mL^{-1}) at day 5 after insemination ($p<0.05$); P4 concentrations of groups 3 and 4 have significant differences with control group (2.69±2.64 and 2.56±1.40 versus 0.81±0.41 ng mL^{-1}, respectively, $p<0.05$) at a day after second injection and groups 4 and 5 were in higher level of P4 concentration than control group at insemination time (3.14±1.9 and 2.89±1.8 versus 0.45±0.19 ng mL^{-1} respectively, $p<0.05$). Pregnancy rate were 0, 50, 45, 10, 30 and 45% for group 1 (control) through 6, respectively.

Key words: Dairy cow, pregnancy rate, serum P4 concentration, synchronization protocols

INTRODUCTION

Synchronisation of oestrus in cattle has been used to facilitate the use of Artificial Insemination (AI) and to minimize the time needed to detect oestrus in cattle (Cavalieri *et al*., 2005). Precise control of the bovine estrous cycle requires control of follicular waves and Corpus Luteum (CL) lifespan (Ahuja *et al*., 2005; Patterson *et al*., 2000). Oestrous synchronization protocols typically employ one of two strategies: 1) administration of a luteolytic agent such as prostaglandin f2α or 2) application of a native or synthetic progestagen to delay the time of estrus following natural or induced luteolysis which may extend the length of the estrous cycle, by CIDR etc (Macmillan and Peterson, 1993; Martinez *et al*., 2002; Kim *et al*., 2003; Mialot *et al*., 2003). Administration of $PG_{F2\alpha}$ analogues induces rapid, premature luteal regression in females with a functional Corpus Luteum (CL) (Kristula *et al*., 1992), followed by spontaneous ovulation (Khanum *et al*., 2006; Ferguson and Galligan 1993; Folman *et al*., 1990). The current and future direction of estrous synchronization is to focus on combining traditional methods of controlling cycle length with the manipulation of follicular development in order to program or select the ovulatory follicle. The immediate goal of controlling both CL function and follicular development is to devise a treatment that will synchronize estrus more precisely and to control the time of ovulation more exactly to allow a single timed insemination without the need for detection of behavioral estrus. The ultimate goal may be to improve the fertility of lactating cows and heifers over that of untreated animals exhibiting a spontaneous estrus. The mode of action of prostaglandins (PG) in the control of ovulation is to induce a premature luteolysis and consequent fall in circulating progesterone concentrations by removal of negative feedback inhibition on the hypothalamus and pituitary, the fall in progesterone concentrations allows a sequence of hormonal and ovarian events that culminate in oestrus and ovulation (Peters, 2005). Recent studies have incorporated GnRH into a synchronization protocol (Are´chiga *et al*., 1998; Burke *et al*., 1996; LeBlanc *et al*., 1998; Pursley *et al*., 1997a; 1995; Schmitt *et al*., 1996; Stevenson *et al*., 1996; Twagiramungu *et al*., 1995) that involves a series of hormone administrations. Ovsynch [synchronization of ovulation (GnRH, day 0; PG_{f2a} day 7; GnRH, day 9; then timed artificial insemination (AI) after second GnRH)] is a management tool that uses

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GnRH and PG_{f2a} to synchronize ovulation, thus allowing control of first and subsequent timed AI in dairy cows (Pursley *et al*., 1997a) and has been shown Pregnancy Rate (PR) to a timed AI following treatment with Ovsynch is similar to AI after observed estrus (Stevenson *et al*., 1999; Britt and Gaska, 1998; De la Sota *et al*., 1998; Burke *et al*., 1996; Pursley *et al*., 1997a; Momcilovic *et al*., 1998; Pursley *et al*., 1997b).

Preparations of GnRH, Human Chorionic Gonadotropin (hCG), LH and P4 are frequently used to treat synchronization of ovulation (Trigg, 2004; Todoroki *et al*., 2001, Tebble *et al*., 2001; Garverick 1997; Osawa *et al*., 1995; Peter 1998; Thatcher *et al*., 1993; Calder *et al*., 1999) but treatment outcomes are highly variable. Recent reports indicate that ovsynch, followed by timed AI performed 16-20 h after the 2nd GnRH treatment yields pregnancy rates of about 25% in cows with occurrence of synchronization of ovulation (Bartolome *et al*., 2000; Fricke and Wiltbank, 1999).

Pharmaceutical control of the reproductive cycle (PCRc) has some advantages and disadvantages. PCRc can remove the estrous detection (Cavalieri *et al*., 2005), also there may be some advantages in shortening the calving to conception interval (Ryan *et al*., 1999), therefore calving interval is reduced and etc. currently the major disadvantage of PCRc is that reproductive performance is highly variable and results vary considerably between herds and between years in the same herd (Padula and MacMillan, 2002).

Hence we conduct an experiment with six different hormone programs in order to determine the best program for shortening the calving to conception interval and pregnancy rate of each program following after timed AI and their hormonal profile position. Here, we particularly used a program for rapid ovulation induction in comparison with other programs of ovulation synchronization.

MATERIALS AND METHODS

Location of farm and date of experiment: This study was conducted on a 1600-Holstein dairy cow in Kermanshah province of Iran. The farm is located in Ravansar region between Latitudes 34° N and 35° N and between Longitudes 46° E and 47° E. The climate of the area is characterized by an average annul of 14.9°C and 537.9 mm mean rainfall per year. The study period lasted almost 3 months from January 2006 to April 2006.

Treatments: One hundred twenty dairy cows (BW = 645±25.245 Kg, Number of parity = 2.8±1.46, Days after Postpartum = 34.6±3.1 and Milk yield = 7358.23±1280.79) were grouped in six groups (Fig. 1):

- In control group each cows received two injections of 500 mg of the prostaglandin $F_{2\alpha}$ ($PG_{f2\alpha}$) analogue, cloprostenol sodium (manufactured by Nasr pharmaceutical company, Fariman, Iran), with 12 days apart.
- In this group 500 mg two injections of Gonadorelin (an analog of GnRH/manufactured by Aburaihan Pharmaceutical Co. Veterinary Division Tehran, Iran) were performed with 9 days apart and also 500 mg $PG_{f2\alpha}$ was injected in day 7.
- At first, all cows were injected by 500 mg GnRH and 7 days later were injected 500 mg $PG_{f2\alpha}$ and then, 2 mg Estradiol Benzoate (EB) (manufactured by Aburaihan pharmaceutical Co. veterinary division Tehran, Iran) was used after 48 h.
- At first, there was an injection of 2 mg EB conjugated with progesterone (manufactured by Aburaihan Pharmaceutical Co, Tehran, Iran) and 7 days later, an injection of 500 mg $PG_{f2\alpha}$ was used and 48 h later 500 mg GnRH was injected.
- Here animals received 2 mg EB conjugated with 100 mg progesterone; after 7 days 500 mg PG_{f2a} was injected then 48 h later 2 mg EB was used.
- In this group 500 mg PG was used per cow and after 24 h animals received 1 mg EB conjugated with 250 IU of Human Chorionic Gonadotropin (hCG) (manufactured by Daroupakhsh pharmaceutical Co. Tehran, Iran).

Estrus detection, insemination and pregnancy determination: In this report, estrous rate have been shown as the percentage of cows in a group that showed

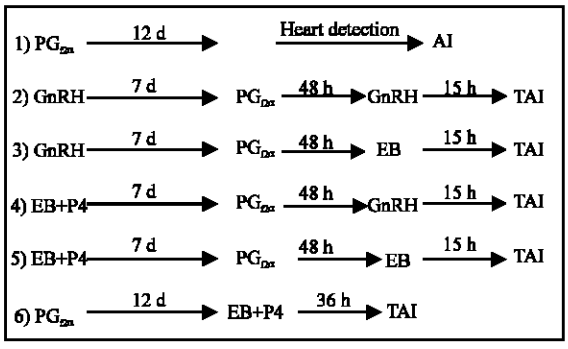

Fig. 1: Schematic representation of synchronization protocols used in each experiment. (PG_{f2a} = prostaglandin $F_{2\alpha}$, GnRH = Gonadotropin Releasing hormone, EB = Estradiol Benzoate, P4 = progesterone, hCG = Human Chorionic Gonadotropin, TAI = Timed Artificial Insemination, d = Day, h = Hour)

estrous activity. Detection of estrous was carried out by heatman as a routine daily activity for monitoring of standing estrous.

Also Artificial Insemination (AI) was carried out as a routine activity via frozen-tawed semen of a same bull.

All cows of group1(control) were inseminated after 72 h but other cows of group 2-5 and cows of group 6 were inseminated after 20 h and after 36 h, respectively. Then animals were examined for pregnancy determination by palpation, 40 days post insemination and pregnancy rate was defined as percentage of cows conceived after AI in a group.

Blood collection and progesterone assay: Blood sampling for determination of serum P4 concentration in group 1 (as control group) through 5 was performed before the first injection (day = 0), day 5 (2 days before second injection), day 8 (a day after second injection) and at insemination time, exactly before AI. But in group 6 that was short-length induction of ovulation, samples were collected before first injection, a day after second injection and exactly before AI. (Because this treatment was short-length induction and there was no sample in 2 days before the second injection).

Blood samples were taken from coccygeal vein. Then blood samples stored stably and allowed to clot at 4°C then centrifuged 2000 g for 10 min and separated serum stored at -20°C until progesterone assay. Serum P4 concentrations were determined using an extract single antibody Radioimmunoassay (RIA).

Data and statistical analysis: The parity number, days in postpartum period and milk yield were the factors that used for grouping of cows. Analysis of serum P4 concentrations between groups was performed via ANOVA procedure in the same time of sampling. The data analysis have expressed as mean±SD that carried out in SPSS program at a p-value less than 5% ($p<0.05$) as significant level using Duncan's multiple range test (Duncan, 1955).

RESULTS

There were no significant differences between experimental animals in number of parity (2.8±1.46), days after postpartum (34.6±3.1) and milk yield (7358.23 ±1280.79) ($p>0.05$).

After RIA of progesterone, it was found out a cow of group4 was in luteal cystic disease, therefore it was removed from trial.

The average of plasma progesterone concentrations on the start day of trial was 2.43±2.16 ng mL^{-1} and there is no difference between experimental animals ($p>0.05$). There is a significant difference in serum P4 concentration of second blood sample of group 4 than control and group 2 and 3 (4.43± 1.50 versus 2.34±1.36, 2.9±1.69, 3.14±2.34 ng mL^{-1}, respectively, $p<0.05$), (Table 1).

Group 3 and 4 had significant differences versus control group in serum p4 concentrations of third blood sampling (2.69±2.64 and 2.56±1.40 versus 0.81±0.41 ng mL^{-1}, respectively, $p<0.05$), (Table 1).

Also, Duncan's multiple range tests shows a higher level of serum p4 concentrations in fourth blood sampling, between group 4 and 5 (3.14±1.9 and 2.89±1.8 ng mL^{-1}, respectively) versus other groups ($p<0.05$) (Table 1).

Number of cows exhibited estrous were 40, 45, 65, 35, 40 and 60% and pregnancy rate were 0, 50, 45, 10, 30 and 45% for group 1(control) through 6, respectively (Table 1).

Table 1: Serum P4 concentrations at various blood sampling, estrus rate and pregnancy rate of experimental groups

	G[1]					
B.S[2]	1(control)	2	3	4	5	6
1	2.24±1.87	2.52±2.35	2.07±1.93	2.72±1.53	2.94±1.48	2.12±2.19
2	2.34±1.36[a]	2.89±1.69[ab]	3.14±2.34[ab]	4.43±1.5[b]	3.74±2.18[ab]	N*
3	0.81±0.41[a]	0.84±0.57[a]	2.69±2.64[c]	2.56±1.49[bc]	1.67±1.06[abc]	1.07±0.85[ab]
4	0.45±0.19[a]	0.54±0.51[a]	0.75±0.38[a]	3.14±1.9[b]	2.89±1.79[b]	1.51±1.5[a]
n	20	20	20	19**	20	20
Estrus rate	40	45	65	35	40	60
Pregnancy rate	0	50	45	10	30	45

Different letter(s) (a, b and c) in each blood samples show a significant difference between groups ($p<0.05$).

1. G = Experimental group

2. B.S = Blood Sample (B.S1: day 0, before the first injection; B.S2: 2 days before second injection; B.S3: In a day after second injection; B.S4: At insemination time, exactly before AI.

*In group6 that was short-length induction of ovulation, samples were collected before first injection, a day after second injection and exactly before AI. (Because this treatment was short-length induction and there was no sample in 2 days before the second injection),

**A cow of group4 was in luteal cystic disease

DISCUSSION

The mean P4 concentration was 2.24 ng mL^{-1} in control group before first injection. Mean P4 concentration was 2.34 ng mL^{-1} 10 days after first injection. Using two injections of $PG_{F2\alpha}$ will bring most females in to heat 2-5 days after the second injection (Khanum *et al*., 2006). The mean P4 concentration was 0.816 ng mL^{-1} one day after second injection of $PG_{F2\alpha}$. This reduction in P4 concentration shows the effect of $PG_{F2\alpha}$ on CL regression. Theoretically, only 55% of cows have sensitive CL to prostaglandin (Taveren, 1992). Therefore, two injections of $PG_{F2\alpha}$ at 11-14 days apart is necessary to bring all cows in synchronization programs (Burk *et al*., 1997). Although estrous synchronization with $PG_{F2\alpha}$ is simple, but has different disadvantages such as variety in pregnancy rate in response to stage of estrous cycle at the time of $PG_{F2\alpha}$ injection (Rayon *et al*., 1998).

Investigation of P4 concentration of second blood samples show presence of ovarian CL in the majority of the animals in group 2 and 3. Response to GnRH treatment is associated with growing phase of dominant follicles (Trimberger, 1948). Predominantly, ovulation is stimulated during the end of growing phase that there are sufficient LH receptors on granulose cells (Webb *et al*., 1999). Reduction in P4 concentration of third blood samples shows CL regression after $PG_{F2\alpha}$ administration and a decrease of less than 1 ng mL^{-1} after second GnRH injection at AI time. Group 3 was similar to group 2 except that EB was used instead of GnRH which induced heat, LH surge and ovulation (Hobson and Hansel, 1972).

In group 4 mean P4 concentrations in the first and second sample were 2.72 and 4.43 ng mL^{-1}, respectively. After $PG_{F2\alpha}$ injection, P4 concentration decreased (2.56 ng mL^{-1}) in third sample. Finally, after GnRH agonist injection, mean P4 concentration increased (3.14 versus 2.56 ng mL^{-1}) in fourth samples before TAI. SerumP4 concentration of the second sample was higher than the first and third ones. These differences show the effect of $PG_{F2\alpha}$ on CL regression. Administration of estradiol in the presence of luteal phase progesterone or an exogenous progesterone source has also been shown to induce follicle atresia and to suppress gonadotropin secretion (Stevenson *et al*., 1984; Michael *et al*., 2000). Treatment with a combination of EB and progestagen has considerable promise for causing follicle atresia and synchronizing follicular development for both estrous synchronization and superovulation (Thatcher *et al*., 2002). Therefore injection of combination of EB and progesterone caused follicular atresia and induction of a new follicular wave and this is the way that in which mean progesterone concentration arrived to 4.43 ng mL^{-1} in second sample.

Administration of GnRH 10, 30 or 50 h after $PG_{F2\alpha}$ likewise reduced the number of cows responding to $PG_{F2\alpha}$. Administration of GnRH 52 h after $PG_{F2\alpha}$ resulted in a better synchronization of ovulation than $PG_{F2\alpha}$ alone, but conception rate was reduced if the GnRH injection was given 60 or 72 h after the $PG_{F2\alpha}$ (Gilbert, 1997). In present study, GnRH injected 48 h after $PG_{F2\alpha}$ treatment and similar to previous works, the estrous and pregnancy rate was low. Increase in P4 concentration after GnRH injection, probably is due to LH secretion and its positive effects on luteinization of granulosa cells.

In group 5 after joint injection of EB and progesterone, the P4 concentration decreased to 3.74 ng mL^{-1} in second sample versus first one and after $PG_{F2\alpha}$ injection decreased to 1.61 ng mL^{-1} in third sample and finally after EB injection, increased in fourth one (2.89 ng mL^{-1}). Similar to group 4 joint injection of EB and progesterone caused follicle atresia then new follicular wave induced and mean P4 concentration arrived to 3.74 ng mL^{-1} in second sample.

The interval from the $PG_{F2\alpha}$ injection to onset of the heat varies depending on the stage of the cycle when $PG_{F2\alpha}$ is administered. Therefore, the age of the CL and status of developing follicle determine time to the onset of the heat following $PG_{F2\alpha}$ injection (Lopez, 2000, Macmillan and Peterson, 1993). Positive feedback loop between estradiol and LH is critical in enabling on ovarian follicle to mature and ovulate and facilitating expression of estrous coincident with ovulation (Schmitt *et al*., 1995). Therefore, injection of EB after $PG_{F2\alpha}$, in this group, caused LH surge and ovulation, therefore mean progesterone concentration before artificial insemination increased.

In group 6, in response to $PG_{F2\alpha}$ injection mean P4 concentrations of second sample decreased versus first one (1.07 vs. 2.12 ng mL^{-1}, respectively) then after joint injection of EB and hCG, serum P4 concentration increased in third sample (1.51 ng mL^{-1}). These results show that $PG_{F2\alpha}$ affect primarily on the corpus luteum; then after joint injection of hCG and EB, mean P4 concentration increases. Treatment with hCG is associated with an increase in the number of large luteal cells and a concomitant reduction in the number of small luteal cells in the CL. Administration of hCG induces a greater increase in plasma progesterone concentration compared to GnRH treatment. This could be due to a combined effect on the original and on the induced CL (Xu *et al*., 1995). In this group, in response to injection of combination of hCG and EB 36 h later (before AI) mean progesterone concentration increased and this demonstrates the effect of hCG and EB on functional CL formation.

Using of two injections of $PG_{F2\alpha}$ with 12 days apart has no pregnancy in the control group in 6 weeks postpartume period, but application of ovsynch in

group 2 induced 45 and 50% in estrus and pregnancy rate, respectively; Pursely *et al.* (1997a) reported use of fixed time AI can result in higher pregnancy rate with no estrus detection. In consistent with Ryan *et al.* (1999), the results of this experiment show that oestrous detection rate and the synchrony of oestrus was increased after administering oestradiol benzoate in group 3, 5 and 6. Previously, Ryan *et al.* (1995) reported administration of oestradiol benzoate at after treatment with $PG_{f2\alpha}$ reduced the interval to oestrus and ovulation that can improve pregnancy rate. Particularly we have carried out a short length program of synchronization that show good results than control group. However it seems these findings warrant further investigation.

REFERENCES

Ahuja, C., F. Montiel, R. Canseco, E. Silva and G. Mapes, 2005. Pregnancy rate following GnRH+$PG_{f2\alpha}$ treatment of low body condition, anestrous Bos taurus by *Bos indicus* crossbred cows during the summer months in a tropical environment. Anim. Reprod., Sci., 87: 203-213.

Are´chiga, C.F., C.R. Staples, L.R. McDowell and P.J. Hansen, 1998. Effects of timed insemination and supplemental β-carotene on reproduction and milk yield of dairy cows under heat stress. J. Dairy Sci., 81: 390-402.

Bartolome, J.A., L.F. Archbald and P. Morresey *et al.*, 2000. Comparison of synchronization of ovulation and induction of estrus as therapeutic strategies for bovine ovarian cysts in the dairy cow. Theriogenolog, 53: 815-825.

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

Burke, J.M., R.L. de la Sota, C.A. Risco, C.R. Staples, E.J.P. Schmitt 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.

Burke, C.R., M.P. Boland, K.L. Macmillan, 1997. Ovarian response to progesterone and estradiol benzoate administered intravaginally during diestrous in cattle. Anim. Rep. Sci., 55: 23-33.

Calder, M.D., B.E. Salfen, B. Bao, R.S. Youngquist and H.A. Garverick, 1999. Administration of progesterone to cows with ovarian follicular cysts results in a reduction in mean LH and LH pulse frequency and initiates ovulatory follicular growth. J Anim. Sci., 77: 3037-3042.

Cavalieri, J., A.R. Rabiee, G. Hepworth and K.L. Macmillan, 2005. Effect of artificial insemination on submission rates of lactating dairy cows synchronised and resynchronised with intravaginal progesterone releasing devices and oestradiol benzoate. Anim. Reprod. Sci., 90: 39-55.

De la Sota, R.L., J.M. Burke, C.A. Risco, F. Moreira, M.A. DeLorenzo and W.W. Thatcher, 1998. Evaluation of timed insemination during summer heat stress in lactating dairy cattle. Theriogenology, 49: 761-770.

Duncan, D.B., 1955. Multiple range and multiple F-tests. Biometrics, 11: l-42.

Fricke, P.M., M.C. Wiltbank, 1999. Effect of milk production on the incidence of double ovulation in dairy cows. Theriogenology, 52: 1133-1143.

Ferguson, J.D. and D.T. Galligan, 1993. Prostaglandin synchronization programs in dairy herds (Part I). Compend. Contin. Educ. Pract. Vet., 15: 646-655.

Folman, Y., M. Kaim, Z. Herz and M. Rosenberg, 1990. Comparison of methods for the synchronization of estrous cycles in dairy cows. 2. Effects of progesterone and parity on conception. J. Dairy Sci., 73: 2817-2825.

Garverick, H.A., 1997. Ovarian follicular cysts in dairy cows. J. Dairy Sci., 80: 995-1004.

Gilbert, R.O., 1997. Reproduction opportunities and challenges. Cornell Vet., 82: 11-14.

Hobson, W.C. and W. Hansel, 1972. Plasma LH levels after ovariectomy, corpus luteum removal and etradiol administration in cattle. Endocrinol., 91: 185-190.

Khanum, S.A., M. Hussain and R. Kausar, 2006. Manipulation of estrous cycle in Dwarf goat (*Capra hircus*) using estrumate under different management conditions. Anim. Reprod. Sci., 92: 97-106.

Kim, I.H., G.H. Suh and D.S. Son, 2003. Aprogesterone-based timed AI protocol more effectively prevents premature estrus and incomplete luteal regression than an Ovsynch protocol in lactating Holstein cows. Theriogenology, 60: 809-817.

Kristula, M., R. Bartholomew, D. Galligan and C. Uhlinger, 1992. Effects of a prostaglandin F_{2a} synchronization program in lactating dairy cattle. J. Dairy Sci., 75: 2713-2718.

Lauderdale, J.W., B.E. Seguin, J.N. Stellflug, J.R. Chenault, W.W. Thatcher, C.K. Vincent and A.F. Loyancano, 1974. Fertility of cattle following $PG_{f2\alpha}$ injection. J. Anim. Sci., 38: 964-967.

LeBlanc, S.J., K.E. Leslie, H.J. Ceelen, D.F. Kelton and G.P. Keefe, 1998. Measures of estrus detection and pregnancy in dairy cows after administration of gonadotropin-releasing hormone within an estrus synchronization program based on prostaglandin $F_{2\alpha}$. J. Dairy Sci., 81: 375-381.

Lopez, F-Gatius, 2000. Reproductive performance of lactating dairy cows treated with cloprostenol, hCG and estradiol benzoate synchronization of estrous followed by timed artificial insemination. Theriogenology, 54: 551-558.

Macmillan, K.L. and A.J. Peterson, 1993. A new intravaginal progesterone releasing device for cattle (DIDR-B) for estrous synchronization, increasing pregnancy rates and the treatment of postpartum anestrous. Anim. Reprod. Sci., 33: 1-25.

Martinez, M.F., J.P. Kastelic, G.P. Adams, B. Cook, W.O. Olson and R.J. Mapletoft, 2002. The use of progestins in regimens for fixed-time AI in beef cattle. Theriogenology, 57: 1049-1059.

Mialot, J.P., F. Constant, P. Dezaux, B. Grimard, F. Deletang and A.A. Ponter, 2003. Estrus synchronization in beef cows: Comparison between GnRH+PGF2+GnRH and PRID+PGF2+eCG. Theriogenology, 60: 319-330.

Michael, J., R. James, E. Kinder, K. Fike, F. Kojima, D. Colin, R. Ogle and C.R. Bunt, 2000. Recent advances in bovine reproductive endocrinology and physiology and their impact on drug delivery system design for the control of the estrous cycle in cattle. Adv. Drug Deliv. Rev., 50: 277-320.

Momcilovic, D., L.F. Archibald, A. Walters, T. Tran, D. Kelbert, C. Risco and W.W. Thatcher, 1998. Reproductive performance of lactating dairy cows treated with gonadotropin-releasing hormone (GnRH) and/or prostaglandin $F_{2\alpha}$ ($PG_{f2\alpha}$) for synchronization of estrus and ovulation. Theriogenology, 50: 1131-1139.

Osawa, T., T. Nakao and M. Kimura *et al.*, 1995. Fertirelin and buserelin compared by LH release, milk progesterone and susbsequent reproductive performance in dairy cows treated for follicular cysts. Theriogenology, 44: 835-847.

Padula, A.M. and K.L. MacMillan, 2002. Reproductive responses of early postpartum dairy cattle to continuous treatment with a GnRH agonist (deslorelin) for 28 days to delay the resumption of ovulation. Anim. Reprod. Sci., 70: 23-36.

Patterson, D.J., S.L. Wood, F.N. Kojima and M.F. Smith, 2000. Current and Emerging Systems to Synchronize Estrus. UNAM (Ed.), VIII Curso Internacional de Reprod. Bovina, M´exico, pp: 97-110.

Peter, A.T., 1998. Infertility Due to Abnormalities of the Ovaries. In: Youngquist, R.S. (Ed.), Current Therapy in Large Animal Theriogenology. Philadelphia: WB Saunders, pp: 349-354.

Peters, A.R., 2005. Veterinary clinical application of GnRH-questions of efficacy. Anim. Reprod. Sci., 88: 155-167.

Pursley, J.R., M.O. Mee and M.C. Wiltbank, 1995. Synchronization of ovulation in dairy cows using $PG_{f2\alpha}$ and GnRH. Theriogenology, 44: 915-923.

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

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

Ryan, D.P., S. Snijders, A. Aarts and K.J. O'Farrell, 1995. Effect of estradiol subsequent to induced luteolysis on development of the ovulatory follicle and interval to estrus and ovulation. Theriogenology, 43: 310. Abstr.

Rayon, A.A., G. Evargas, C.A.Valdez and D.B. Marcial Jr., 1998. Estrous synchronization using progesterone intravaginal device with or without estradiol in dairy cattle. Phil. J. Vet. Med., 36: 35-39.

Ryan, D.P., J.A. Galvin and K.J. O'Farrell, 1999. Comparison of oestrous synchronization regimens for lactating dairy cows. Anim. Reprod. Sci., 56: 153-168

Schmitt, E.J.P., M. Drost, T. Diaz, C. Roomes and W.W. Thatcher, 1995. Effect of a Gonadotropin-Releasing Hormone agonist on follicle recruitment and pregnancy rate in cattle. J. Anim. Sci., 74: 154-160.

Schmitt, 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., M.K. Schmidtand E.P. Call, 1984. Gonadotropin-releasing hormone and conception of holsteins. J. Dairy Sci., 67: 140-145.

Stevenson, J.S., Y. Kobayashi, M.P. Shipka and K.C. Rauchholz, 1996. Altering conception of dairy cattle by gonadotropin-releasing hormone preceding luteolysis induced by prostaglandin $F_{2\alpha}$. J. Dairy Sci., 79: 402-410.

Stevenson, J.S., Y. Kobayashi and K.E. Thompson, 1999. Reproductive performance of dairy cows in various programmed breeding systems including Ovsynch and combinations of gonadotropin releasing hormone and prostaglandin $F_{2\alpha}$. J. Dairy Sci., 82: 506-515.

Taverne, M.A.M., 1992. Physiology of Parturition. Anim. Reprod. Sci., 28: 433-440.

Tebble, J.E., M.J. O'Donnell and H. Dobson, 2001. Ultrasound diagnosis and treatment outcome of cystic ovaries in cattle. Vet. Rec., 148: 411-413.

Thatcher, W.W., M. Drost and J.D. Savio *et al*., 1993. New clinical uses of GnRH and its analogues in cattle. Anim. Reprod. Sci., 33: 27-49.

Thatcher, W.W., F. Moreina, S.M. Pancarci, J.A. Bartolome and J.E.P. Santos, 2002. Strategies to optimize reproductive efficiency by regulation of ovarian function. Dom. Ani. End., 23: 243-254.

Todoroki, J., H. Yamakuchi and K. Mizoshita *et al*., 2001. Restoring ovulation in beef donor cows with ovarian cysts by progesterone-releasing intravaginal silastic devices. Theriogenology, 55: 1919-1932.

Trigg, T., 2005. Applications of GnRH in the control and management of fertility in female animals. Anim. Reprod. Sci., pp: 88: 141-153.

Trimberger, G.W., 1948. Breeding efficiency in dairy cattle from artificial insemination at various intervals before and after ovulation. Nebr. Agric. Exp. Stat. Res Bull., 153: 3-25.

Twagiramungu, H., L.A. Guilbault and J.J. Dufour, 1995. Synchronization of ovarian follicular waves with a gonadotropin-releasing hormone agonist to increase the precision of estrus in cattle: A review. J. Anim. Sci., 73: 3141-3151.

Webb, R., B.K. Campbell, H.A. Garverick, J.G. Gong, C.G. Gutierrez and D.G. Armstrong, 1999. Molecular mechanisms regulating follicular recruitment and selection. J. Rep. Fertil., pp: 33-48.

Xu, Z.Z., L.J. Burton and K.L. Macmillan, 1995. Reproductive performance of lactating dairy cows following estrous synchronization regimens with $Pg_{I2\alpha}$ and progesterone. Theriogenology, 47: 687-701.