PhD thesis

Effect of prostaglandin treatment on the corpus luteum, plasma progesterone concentration and the largest follicle in dairy cow

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Budapest
2005

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Contents

List of abbreviations

AI	Artificial Insemination
ANOVA	Analysis of variance
CL	Corpus Luteum
CL0	Absence of Corpus Luteum
CL1	Growing Corpus Luteum
CL2	Mid-cycle Corpus Luteum
CL3	Regressing Corpus Luteum
CR	Conception Rate
D	Dissection of ovaries
ET	Endothelin
F	Follicle
GLM	General Linear Model
IM	Intramuscular
IV	Intravenous
LLC	Large Luteal Cells
LH	Luteotrop Hormon
MP	Manual Palpation
NEB	Negative Energy Balance
PG	Prostaglandin
+PV, –PV	Predictive Values
P4	Progesteron
PR	Pregnancy Rate
RIA	Radio Immuno Assay
RP	Rectal Palpation
SC	Subcutaneous
Se	Sensitivity
Sp	Specificity
SLC	Small Luteal Cells
THAM	Tromethamine Salt
US	Ultrasonography

Introduction

Reproductive efficiency is a critical component of a successful dairy herd management, whereas a reproductive inefficiency is one of the most costly problems facing the dairy industry today. Fertility in lactating dairy cows has decreased from 66% in 1951, to about 50% in 1975, and to about 28% (Kristula et al., 1992) to 42% (Archbald et al., 1993) currently. Therefore the fertility of dairy cows is a growing concern. Calving interval is a major component which involves the days from calving to the initiation of the next pregnancy, usually referred as open days, and the fixed effect of gestation length. Open days depend on the days from calving to the first insemination or mating and fertilization, and associated with conception rate. It is a great problem in Hungary, because the calving interval has been increasing from 1970 till nowadays. Risco et al. (1995) and Thatcher et al. (1993) emphasized that "to be effective in any drug therapy that shortens the calving interval and to induce ovulation must go hand in hand with good reproductive management and excellent estrus detection". The synchronised ovulation regimes reduce the time required for estrus detection but about 60% of synchronized cows do not conceive at first service. The importance of good estrus detection was also emphasized by Kinsel and Etherington (1998) who surveyed 45 herds using conventional detection of estrus or GnRH and/or Progestogens in their breeding program. The effectiveness of estrus detection, and the conception rate had a great impact on the calving interval. Nebel et al. (1987) reported that detection of estrus was a problem in 30% of the herds studied with up to 46% of the cows inseminated when progesterone (P4) concentration in the milk was high. The latter results in low conception rates, and insemination of pregnant cows can induce embryonic or fetal mortality. Both events increase the calving interval.

The success of estrous induction with PGF2α depends on the presence of a functional corpus luteum. Traditionally, rectal palpation (RP) of the reproductive tract is used to identify cows with a corpus luteum eligible for PGF2α treatment. Overall, a 77% (Ott et al. 1986), to 79 % (Archbald et al. 1993) agreement between the diagnosis of a CL by an experienced palpator and the progesterone concentrarion was found. It was concluded that RP may be inadequate for identifying cows with a mature CL for induction of estrus by PGF2α treatment.

The detection of a CL by ultrasonography proved 96 per cent accurate, as judged by milk P4 concentration (> 5 ng/ml) (Smith et al., 1998). An accuracy of 100% would not be expected

because it has a period of two days at the end of the cycle, when the corpus luteum remains ultrasonographically visible (without a significant reduction in size) despite the plasma P4 concentration falling to basal values (Ribadu et al., 1994).

However, there is a great variation in time of oestrus/ovulation over periods of 5 days after injection of PGF2α due to the fact that the time of onset of estrus/ovulation is mainly dependent on the follicular status when luteolysis is induced (Odde 1990; Lucy et al. 1992; Roche and Mihm 1996). This great variation can also be confirmed by higher pregnancy rate achieved when A.I. is performed after detected estrus than that after timed A.I. (Archbald et al. 1992; Lucy et al. 1986; Stevenson et al. 1987).

Various attempts have been made to overcome this variability in response to prostaglandin treatments. The administration of other hormones in conjunction with prostaglandin, such as progesterone, oestradiol benzoate, hCG, GnRH (Deletang 1975; De Rensis and Peter 1999; Pursley et al. 1996) were attempted. There were a better degree of synchronization but the pregnancy rate was similar to that of untreated cows.

The purpose of the thesis is to survey the lifespan of the CL during the estrus cycle, the changes of the P4 concentration, the diagnosis of ovarian structures by means of rectal palpation and ultrasonography, and to discuss the synchronization techniques of estrus by inducing luteolysis with prostaglandin treatments (effect of different doses and techniques, and application modes of PGF2α treatments, and failure of luteolysis).

The main objective of our examinations was to study particularly the effects of prostaglandin treatment on the corpus luteum, the largest follicle, the progesterone concentration, and the time of detected oestrus and/or ovulation in dairy cow, using different doses (0 mg, 25 mg, vs. 35 mg), different types (natural vs. synthetic) and different number (once vs. twice 8 h apart) of prostaglandin treatments from the day of treatment (Day 0).

Further objective of our examinations was to negotiate the parameters (the area of CL, and the largest follicle, time of ovulation, pregnancy rate in relation to the time of ovulation) of treated (single dose) and non treated cows when the Day 0 was day of AI.

Summary

The review survey the lifespan of the CL during the estrus cycle, the luteolytic mechanisms in the bovine corpus luteum, the changes of the progesterone (P4) concentration, the diagnosis of ovarian structures by means of rectal palpation (RP) and ultrasonography, when comparing the accuracy (with P4 concentration) of the detection of a CL by rectal palpation, and ultrasonography. It is concluded that RP may be inadequate for identifying cows for any kind of treatment.

The review discusses the synchronization techniques of estrus by inducing luteolysis with prostaglandin treatments. Synchronization with a single injection of PGF2α did not control the time of AI, because estrus detection continued to be necessary. When timed AI after PGF2α in lactating dairy cows was examined, pregnancy rates per AI were substantially lower than those for AI after a detected estrus. Much of the variation in time to ovulation was probably due to the variation in stage of growth of the preovulatory follicle at the time of PGF2α treatment. Finaly the application methods, different doses and different number of PGF2α treatments with different intervals, and failure of luteolysis are discussed.

In the first experiment the effects of different doses (0 mg, 25 mg vs 35 mg) of prostaglandin treatments from the day of treatment (Day 0) were examined. The percentage changes relative to the corpus luteum area decreased, and the percentage changes relative to the largest follicle area increased faster, and even the oestrus started sooner in cows treated with 35 mg PGF2α than in those treated with 25 mg PGF2α. However, these differences between groups were not statistically significant. At the same time, the decrease in the percentage changes relative to the area of corpora lutea and to the concentrations of P4 was statistically significant in both groups.

In the second experiment treatment of dairy cows with 2 luteolytic dosages of PGF2α or its synthetic analogue at an 8-h interval resulted in more cows (non-significantly) (18 vs. 21) being observed in oestrus within 5 d after treatment and having significantly higher conception rate (27,8% vs. 66,6%) than with 1 treatment. Further studies in progress should confirm the benefit of 2 prostaglandin treatments in a larger scale. At the same time, the type and the number of prostaglandin treatments had no effect on the incidence of ovulations after oestrus, the number of ovulations without oestrous signs, the number of cows without oestrus and ovulation, and the average time from treatment to oestrus.

In the third experiment the time of ovulation was examined after detected oestrus and A.I. (Day 0) in prostaglandin treated and non-treated dairy cows. Large variations in the area of the CL were detected in the prostaglandin treated and untreated cows. The areas of the largest follicles in treated cows were somewhat smaller during the experiment, than those in untreated cows however those differences between the groups and within the groups were not statistically significant. The area of the largest follicle in cows with no ovulation also did not differ significantly. Some of the cows in treated and non-treated groups did not ovulate at all during the experiment. The mean area of the ovulatory follicle on the day before ovulation was somewhat greater but not significantly, if ovulation occurred later regarding to AI. The overall conception rate was > 50% in both groups, but when the cows ovulated too early or too late in relation to the time of AI the conception rate was significantly lower, therefore determination of the optimal time for AI is of great practical importance. If ovulation does not occur within two days after AI second AI may be performed. Further studies are needed to evaluate the benefit of the second AI.

Összefoglaló

A bevezetőben áttekintjük a sárgatest fejlődési fázisait, a luteolízist, a progeszteron szint változásait, a petefészek képleteinek diagnózisát rektális vizsgálattal és ultrahanggal. A sárgatest ultrahangos és rektális felismerhetőségét összehasonlítva (alapul véve az ugyanakkor mért P4 szintet), megállapíthatjuk hogy a rektális vizsgálatnál lényegesen pontosabb az ultrahangos vizsgálat.

A bevezetőben bemutatásra kerülnek a prosztaglandinokkal végzett ivarzás szinkronizálási módszerek. Az általános adaggal végzett PGF2α kezelések legnagyobb hátránya, hogy nem tudjuk behatárolni sem az ivarzás, sem az ovuláció pontos időpontját, így a meghatározott időben egyszer végzett termékenyítések után a vemhesülési arány mindig alacsonyabb, mint a megfigyelt ivarzások után végzett termékenyítések esetén. A kezeléstől az ovulációig eltelt időszak főleg a kezeléskori preovulációs tüsző növekedési állapotától függ.

Végül számos PGF2α kezelési módszert tárgyalunk (különböző beadási helyek, különböző adagok, különböző számú kezelés különböző időközökkel), valamint a teljes luteolízis elmaradásának okait.

A saját vizsgálatainkat két részre lehet osztani. Az első részben a különböző adagokkal (0 mg, 25 mg, ill. 35 mg), különböző típusú prosztaglandinokkal (természetes, mesterséges), és különböző számban (egyszer vagy kétszer 8 órás időközzel) végzett kezelések hatásait vizsgáltuk.

Megállapítottuk, hogy a 35 mg PGF2α-val kezelt állatok átlagos sárgatest területe gyorsabban csökkent, valamint a legnagyobb tüszők területe gyorsabban nőtt, ill. az ivarzás bekövetkezte a kezeléstől számítva hamarabb megtörtént, mint a 25 mg-al kezelt állatok esetén, habár a különbségek nem voltak statisztikailag szignifikánsak.

A sárgatestek méretének, valamint a P4 szinteknek a százalékos csökkenése a 0. naphoz (a prosztaglandin kezelés napja) képest azonban szignifikáns eltérést mutatott.

Két szimpla dózissal 8 órás időközzel végzett természetes, ill. szintetikus PGF2α kezelés több ivarzást (nem szignifikánsan), és magasabb termékenyülési arányt (szignifikánsan) eredményezett, mint az egyszeri kezelés.

Ugyanakkor, a prosztaglandin típusa és a kezelések száma nincs befolyással a kezeléstől az ovulációig eltelt időszakra, az ivarzási tünetek nélkül ovuláló állatok számára, az ovuláció és az ivarzási tünetek nélküli állatok számarányára, valamint a kezeléstől az ivarzásig eltelt időszakra.

Saját vizsgálataink második részében az egyszeri szimpla adaggal kezelt, és a nem kezelt tehenek szaporodásbiológiai adatait hasonlítottuk össze, ahol a 0. napként a termékenyítés időpontját vettük alapul.

Nagy egyedi különbségek voltak tapasztalhatóak a sárgatestek méreteiben az egyes vizsgált napokon mind a kezelt, mind a nem kezelt tehenek esetében.

A kezelt állatok tüszőinek átlagos területe nem szignifikánsan, de kisebb volt, mint a nem kezelteké. A nem ovuláló tehenek tüszőinek mérete sem különbözött szignifikánsan az ovuláltakétól.

Az preovulációs tüszők területeinek átlagai, az ovuláció előtti napon annál nagyobbak voltak (nem szignifikánsan) minél később ovuláltak a termékenyítéshez képest.

Az összfogamzási arány 50% felett volt mindkét csoportban, de ha a tehenek túl hamar vagy túl későn ovuláltak a termékenyítés idejéhez képest, a fogamzási arány szignifikánsan kisebb volt. Ebből kiindulva a termékenyítés optimális idejének megtalálása nagy gyakorlati jelentőséggel bír.

Ha az ovuláció nem következik be két napon belül a termékenyítéshez képest, újabb termékenyítésre volna szükség. Ennek alátámasztására újabb vizsgálatok szükségesek.

Chapter 1

Synchronization of estrus with prostaglandin: review

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General introduction

Reproductive efficiency is a critical component of a successful dairy herd management, whereas a reproductive inefficiency is one of the most costly problems facing the dairy industry today. Therefore the fertility of dairy cows is a growing concern. Calving interval is a major component which involves the days from calving to the initiation of the next pregnancy, usually referred as open days, and the fixed effect of gestation length. Open days depend on the days from calving to the first insemination or mating and fertilization, and associated with conception rate. Risco et al. (1995) and Thatcher et al. (1993) emphasized that "to be effective in any drug therapy that shortens the calving interval and to induce ovulation must go hand in hand with good reproductive management and excellent estrus detection". The synchronised ovulation regimes reduce the time required for estrus detection but about 60% of synchronized cows do not conceive at first service. The importance of good estrus detection was also emphasized by Kinsel and Etherington (1998) who surveyed 45 herds using conventional detection of estrus or GnRH and/or P4 in their breeding program. The effectiveness of estrus detection, and the conception rate had a great impact on the calving interval. Nebel et al. (1987) reported that detection of estrus was a problem in 30% of the herds studied with up to 46% of the cows inseminated when progesterone concentration in the milk was high. The latter results in low conception rates, and insemination of pregnant cows can induce embryonic or fetal mortality. Both events increase the calving interval.

The purpose of the review is to survey the lifespan of the CL during the estrus cycle, the changes of the P4 concentration, the diagnosis of ovarian structures by means of rectal palpation and ultrasonography, and to discuss the synchronization techniques of estrus by inducing luteolysis with prostaglandin treatments (effect of different doses and techniques, and application modes of PGF2α treatments, and failure of luteolysis).

1. Physiological events connected with the luteal phase

1.1. Formation of a corpus luteum after ovulation

The corpus luteum (CL) is a temporary endocrine gland that secretes progesterone (P4) to support pregnancy. It develops from the ovarian follicle after ovulation. The CL is controlled by hormones which play a crucial role in providing the signal for luteotropic support during the estrous cycle and pregnancy and for inducing luteolysis at the end of the cycle.

The term CL literally means yellow body. In the cow the yellow colour is originated from the high levels of β carotene, a precursor of the antioxidant vitamin A (Graves-Hoagland et al., 1989; Hurley and Doanne 1989).

Luteotrop hormone (LH) is the major pituitary hormone for regulating the CL in a number of species. During the bovine estrous cycle LH is secreted at low levels except for the large preovulatory surge. This surge stimulates the ovulation of pre-ovulatory follicle and the formation of a CL. The follicle contains an inner avascular layer of granulosa cells surrounded by a basement membrane, a layer of theca interna and an outer layer of theca externa. A number of structural changes used to follow ovulation. The basement membrane breaks down, the vascular theca interna and the granulosa cells invade the follicular cavity. Cells from the granulosa and the theca interna will grow and divide new vessels will proliferate to supply the CL with a vascular network. Blood flow increases as the CL grows (Damber et al., 1987).

The CL contains 2 types of luteal cells, which can be distinguished by size (Koos and Hansel, 1981). In non-pregnant cows small luteal cells range from 10-20 μM in diameter, and they are derived from the theca interna layer of the follicle (Alila and Hansel, 1989). The large luteal cells are ≥25 μM in diameter. In the early stages of the estrous cycle, the large cells develop from the granulosa layer, but in the later stages they also develop from the small cells derived from the theca interna (Hansel et al., 1987). Vascular cells are also present in the CL. They include endothelial cells, which line the capillaries, erythrocytes and various leukocytes including eosinophils, T-lymphocytes and macrophages (Adashi, 1990). Recent studies indicate that vascular cells may play an important role in regulating CL function by releasing various chemical messengers, which function as local hormones to stimulate or inhibit P4 secretion. (Milvae et al., 1996.)

1.2. Luteolytic mechanisms in the bovine corpus luteum

The term prostaglandin (PG) appeared in the literature in the early 1930's and was applied by von Euler as reported by Speroff and Ramwell (1970), to a new group of physiologically active substances extracted from ovine vesicular glands. PGs are analogues of the hypothetical 20 carbon prostanoic acid. The PG is subdivided into A-I series according to the chemical structure of the cyclopentane ring. (Rudas and Frenyó, 1995).

In the absence of fertilization, the CL undergoes morphological and functional regression. This process, called luteolysis, is characterized by a cessation of P4 production and a

breakdown of cellular components, including reduction of vascular supply, proliferation of connective tissue, increased cellular disorganization, and degeneration and phagocytosis of the luteal cells (Carlson et al., 1982). PGF2α secreted by the endometrium is widely believed to be the major endogenous luteolysin in domestic ruminants (McCracken and Schramm, 1988). The pulsatile nature of uterine secretion of PGF2α is the result of a positive feed back loop in which luteal oxytocin binds to endometrial receptors to stimulate the release of PGF2α at intervals of approximately 6 hours during the process of luteolysis (Flint et al., 1992; Silvia et al., 1991). A number of intracellular mechanisms by which PGF2α and its analogues causing luteolysis have been observed in domestic ruminants based on the experiments conducted in vitro and in vivo. These include a dramatic decrease in luteal blood flow (Nett et al., 1976), changes in membrane permeability (Carlson et al., 1982), altered activity of steroidogenic enzymes (Caffrey et al., 1979; Rao et al., 1984), inhibition of lipoprotein stimulated steroidogenesis (Pate and Condon, 1989; Wiltbank, 1990), alteration of nuclear chromatin conformation (Chegini et al., 1991), decrease in the number of small lutein cells (Braden et al., 1988), release of luteal oxytocin (Flint and Sheldrick, 1982), and a decrease in luteal prostacyclin (Milvae and Hansel, 1983). Reported effects of PGF2α on signal transduction include a decrease in gonadotropin receptors (Rao et al., 1984), an uncoupling of the LH receptor and adenyl cyclase (Fletcher and Niswender, 1982; Rodgers, 1990), stimulation of phospholipase C activity (Davis et al., 1988; Jacobs et al., 1991), increase of inositol triphosphate and intracellular free calcium (Alila et al., 1989; Davis et al., 1989; Duncan and Davis, 1991) and alteration of protein kinase C activity (Orwig et al., 1994). PGF2a receptors have been localized to the plasma membrane of large luteal cells (Powell et al., 1976). According to Davis et al. (1988), PGF2α increases phospholipase C activity in both small and large luteal cells led to the suggestion that small luteal cells may have sufficient receptors to respond directly to PGF2α. However, there are interactions between endothelial cells and luteal cells. Uterine PGF2α release induces vasoconstriction within the luteal vasculature resulting in hypoxia and endothelin-1 (ET-1) release from resident endothelin cells. ET-1 inhibits basal and LH-stimulated P4 biosynthesis in small and large luteal cells directly via ET-1 action on ET receptors. Additionally, ET-1 alters arachidonic acid metabolism resulting in a net increase in the production of PGF2α and a reduction in the proposed luteotropin, PGI2, by bovine luteal tissue (Stephenson et al., 1993; Vane and Botting 1990). Removal of PGF2α through active or passive immunization prevents spontaneous luteal regression. Treatment of animals with inhibitors of prostaglandin synthesis

has also been shown to block spontaneous luteolysis in domestic ruminants (Milvae et al., 1986).

1.3. Changes in progesterone (P4) concentrations during estrous cycle

The estrous cycle can be divided into three endocrine periods: pre-gonadotropin surge (Days 19-0), post-gonadotropin surge (Days 1-3), and luteal phase (Days 4-18) (Hansel and Convey, 1983). During the pre-gonadotropin surge, concentration of P4 rapidly declines, and reaching its basal level within a period of two days at the end of the cycle, when the CL still remains ultrasonographically visible. There is a significant correlation between the area of the luteal tissue and plasma P4 concentration during the second half of the cycle in animals which can not be pregnant (r=0,77), but for unknown reasons it has not been observed for heifers who became pregnant following that cycle (r=0,33) (Kastelic et al., 1990b). It has also been demonstrated that, after luteolysis, the physical regression of the CL is a slower process than the decrease in P4 production. The area of the CL measured by means of ultrasonography and P4 concentration decreases daily by 20% and 28%, respectively (Kastelic et al., 1990b).

During the post-gonadotropin surge (called as metestrus) plasma concentration of P4 remains low (Hansel and Convey, 1983).

The luteal phase begins when the new CL secretes significant concentration of P4, which generally exceed 1 ng/ml by Day 4 of the cycle (Rahe et al., 1980). According to Ricoy et al. (2000) <1 ng/ml serum P4 levels indicates the presence of follicular phase of the estrous cycle and possibly the presence of true estrus and concentrations of >1ng/ml P4 indicates the presence of luteal phase and absence of estrus.

White and Sheldon (2001) showed that $\geq$5 ng/ml milk P4 concentrations indicated the presence of active luteal tissue and cows in estrus had <5 ng/ml milk P4 concentrations. However, in that study the P4 concentration was $\geq$5 ng/ml in 19,7% of the samples taken during estrus. This is greater than that expected during estrus. Similar proportions of animals with milk P4 concentrations in excess of those expected during estrus have been reported elsewhere (Nebel et al., 1987). A luteal structure >11 mm usually correlates with the milk progesterone concentration $\geq$5 ng/ml (Sprecher et al., 1989).

Plasma P4 reaches maximal concentrations by Days 8 to 10 (Niswender et al., 1994). The CL develops, and plasma P4 concentrations rise to a plateau of 6-10 ng/ml from Days 7 to 18 (Rahe et al., 1980). A strong and a significant correlation also exist between the area of the luteal tissue and plasma concentration of P4 during the first half of the estrous cycle in non-pregnant animals (r=0,73) and in animals which become pregnant (r=0,85) (Hanzen et al.,

2000). A positive correlation was also observed between ultrasonographically measured CL diameter and plasma P4 concentrations during spontaneous development and regression of CL in heifers (Kastelic et al., 1990b). The size of the CL determined by ultrasonography is strongly correlated (r=0,68-0,85) to milk P4 concentrations (Sprecher et al., 1989; Rajamahendran and Taylor, 1990; Ribadu et al., 1994). The correlation coefficients between the area of the CL and the milk P4 concentration during the luteal development (Days 2-8) were r=0,69 (P<0,0001) and r=0,75 (P<0,0001) for the CL with and without a cavity, respectively, while, during the luteal regression (Days -6 to 0 relative to the next ovulation), their coefficients were r=0,73 (P<0,0001) and r=0,77 (P<0,0001), respectively. By this way there was no significant difference between the corpora lutea with or without a cavity (Son et al., 1995).

During summer, the luteal function is suppressed. The plasma P4 concentration between Days 6 and 18 of the estrous cycle was found to be significantly (P<0,05) lower (4,8±0,9 ng/ml) than during spring (7,4±0,9 ng/ml) (Howell et al., 1994). At the same time Imtiaz-Hussain et al. (1992) observed lower concentrations of luteal phase P4 during summer in Holstein cows (a heat-intolerant breed) than in Jersey cows (a more heat-tolerant breed).

2. Diagnosis of ovarian structures by means of rectal palpation and ultrasonography

2.1. Follicles

The follicles appear as dark, sometimes delineated, anechogenic structures, surrounded by a fine wall and with a diameter usually <25 mm. Due to the lack of attenuation of the ultrasound wave, a hyperechogenic border is usually seen at the distal zone of the follicle. Ultrasonography will, however, only show the cavity, thus the real diameter of the follicle is often underestimated by 2-3 mm (Quirk et al., 1986). The presence of several follicles or a CL can cause compression of the follicles, making them appear irregular in outline (Pierson and Ginther, 1988b). It is now well established that two or three waves of follicular development occur during the majority of bovine cycles. However, a small proportion of cycles exhibit only one or alternatively four waves per cycle. Cows differ in the relative proportion of the cows exhibiting two-wave versus three-wave cycles. Transrectal ultrasonic imaging reveals that most estrous cycles have two (Ginther et al., 1989a) or three (Savio et al., 1988; Sirois and Fortune, 1988) follicular waves. A wave of follicular development in cow is characterized by synchronous growth of a number of small follicles followed by selection of a dominant

follicle and subsequent regression of the subordinate follicles (Savio et al., 1988; Sirois and Fortune 1988; Ginther et al., 1989a,b; Knopf et al., 1989). Generally the first dominant follicle of the estrous cycle is detectable as one of a cohort of 2-5 mm follicles that are present on the day after ovulation. It is selected between Days 2 and 3 of the cycle and becomes dominant between Days 4 and 5. The dominant follicle reaches its maximum diameter of 13-16 mm between Days 6 and 7 of the cycle. This is followed by a period of relative stability between Days 6 and 10. Finally it decreases in size and is no longer identifiable by Day 15. (Sunderland et al., 1994). During the normal estrous cycle the first dominant follicle does not ovulate. In cow with two follicular waves, if the second dominant follicle is recruited by Day 10 of the cycle it will ovulate 11 days later. In cows with 3 follicular waves (the second dominant follicle is not recruited by Day 12), the ovulatory follicle emerges around Day 16 and ovulates 7 days later.

2.2. Corpus luteum

A review article by Ott et al. (1986) summarizes the findings of multiple studies comparing rectal palpation (RP) with P4 concentrations. There is a 77% to 79 % agreement between the diagnosis of a CL by an experienced palpator and P4 concentration (Ott et al., 1986; Archbald et al., 1993). Ott et al. (1986) concluded that RP may be inadequate for identifying cows for any kind of treatment. The detection of a CL by ultrasonography proved 96 per cent accurate, as judged by milk P4 concentration (> 5 ng/ml) (Smith et al., 1998). An accuracy of 100% would not be expected because it has a period of two days at the end of the cycle, when the corpus luteum remains ultrasonographically visible (without a significant reduction in size) despite the plasma P4 concentration falling to basal values (Ribadu et al., 1994). As Tables 1 and 2 show that the detection of a CL by means of ultrasonography using 7.5 linear-array transducer is more precise, than by rectal palpation. On Days 1-7 of the estrous cycle the size of the CL increases (Kastelic and Ginther, 1991; Assey et al., 1993; Kastelic et al., 1990a), while between Days 8-14 it reaches its maximal size (Kastelic et al., 1990a; Tanabe and Hahn, 1984), which decreases from Day 14 to ovulation (Kastelic et al., 1990a).

Combined for interovulatory intervals and pregnancies, the CL was first detected (an echogenic area clear enough to be measured was visible on day of ovulation) on Day 0 (ovulation) (73%), Day 1 (89%), Day 2 (94%), Day 3 (99%), Day 4 (100%), respectively (Kastelic et al, 1990a). The outline of the midsaggital section of the CL was round or oval. A well-defined border was visible after approximately Day 3, the new CL was then clearly demarcated from the ovarian stroma and follicles and was easily identified (Kastelic et al,

1990a). CL has a mean width of 14 mm and a mean length between 18 and 21 mm when they first become easily detectable on the third day after ovulation (Pierson and Ginther, 1984; Kähn, 1986). Then it grows 1 mm in width and 2 mm in length per day, and reaches their maximum size of about 20 × 30 mm by Days 8 to 10 post ovulation (Quirk et al., 1986). After natural or induced luteolysis the largest diameter of the CL rapidly began to decrease to below 23 mm (Quirk et al., 1986).

The developing corpus haemorrhagicum (CL1) is a poorly delineated, irregular, greyish-black structure with several echogenic spots within the ovary (Pierson and Ginther, 1984; Edmondson et al., 1986; Omran et al., 1988; Pieterse, 1989; Kastelic et al., 1990a; Ribadu et al., 1994). The echogenicity of luteal structures increases during diestrus. A mature CL appears as a greyish echogenic area with a line of demarcation visible between it and the ovarian stroma.

The ultrasonographic appearance of the CL is very similar in pregnant and non-pregnant animals (Pierson and Ginther, 1984; Edmondson et al., 1986; Omran et al., 1988; Pieterse, 1989; Kastelic et al., 1990a; Ribadu et al., 1994), and it is impossible to distinguish a particular day of diestrous (Pieterse, 1989). According to a number of studies (Kito et al.,1986; Pierson and Ginther, 1987; Kastelic et al., 1990a), the ultrasonographic appearance of the central cavity of a fluid-filled corpus luteum is similar to that of a follicle (Kahn and Leidl, 1989). Usually, it is less regular, surrounded by luteal tissue, rounded and presented more often highly echogenic bands or echogenic spots corresponding respectively to fibrin-like strands and accumulations of haemolysed blood (Pierson and Ginther, 1987). The formation of fluid-filled corpora lutea has been hypothesized to be due to a premature closing of the ovulation site (McEntee, 1990). In the absence of serial ovarian examinations to confirm ovulation, it may be difficult to determine if a luteal structure with a large fluid-filled central cavity originated from an ovulated follicle (cystic corpus luteum: corpora lutea with fluid-filled cavities of variable sizes and remaining longer than 7 days) or an anovulatory follicle (luteal cyst) (McEntee, 1958). The central cavity, or lacuna, of the CL may have a diameter of between 2 and 22 mm (Kito et al., 1986). Kastelic et al. (1990a) reported that 35% of these cavities have a diameter >10 mm, with 52% ranging from 6 to 10 mm, and 13% from 2 to 5 mm with no difference between pregnant and non-pregnant animals. This cavity may regress or persist through the cycle. Generally, the diameter is the greatest on Day 10 after ovulation (Kastelic et al., 1990b). The largest cavities used to be detectable for a longer period of time. Cavities >10 mm take more than 21 days to disappear (Kito et al., 1986). On average, smaller cavities regress within 1 week (Kito et al., 1986). In non-pregnant cows, the

amount of luteal tissue is independent of the diameter of the lacuna (Kastelic et al., 1990a). Previous ultrasound study (Kastelic et al., 1990) indicated that central luteal cavities usually regressed while the corpus luteum was being maintained. Occasionally, loss of a large cavity was hastened in association with luteolysis. The presence or the size of the cavity has no statistically significant influence on serum P4 concentration (Kito et al., 1986; Quirk et al., 1986; Kastelic et al., 1990b; Ribadu et al., 1994), but Garcia and Salaheddine (2000) found in nonpregnant heifers the progesterone concentration of CL without cavity was higher (no significant difference) than nonpregnant heifers in all three (small 2-5 mm, medium 6-10 mm, and large >10 mm) cavity categories. Kaneda et al. (1980) did not find statistically significant influence between the duration of return to estrus and the presence or the size of the cavity, similarly to Kito et al. (1986) between the potential of the animal to become pregnant and the presence or the size of the cavity. However, the luteal cavity areas of pregnant heifers was significantly smaller than those of nonpregnant heifers in all three (small 2-5 mm, medium 6-10 mm, and large >10 mm) cavity categories (Garcia and Salaheddine, 2000). Moreover, there is also no tendency for a cavity to recur from one cycle to another in the same animal (Kito et al., 1986; Kastelic et al., 1990a).

In a regressing CL, the line of demarcation is faint due to the slight difference in echogenicity between tissues (Pierson and Ginther, 1984; Kastelic et al., 1990a). The CL from the previous ovulation can be detected until Day –2 (98%), Day –1 (84%), Day 0 (63%), Day 1 (38%), Day 2 (24%), Day 3 (12%), Day 4 (8%), Day 5 (6%), Day 6 (2%), Day 7 (0%) relative to the second ovulation (Kastelic et al., 1990).

Season does not affect growth, maximum size, or regression rate of the CL (Howell et al., 1994; Kastelic et al., 1990a; Kastelic et al., 1990b). Central luteal cavities can be observed at similar rates during both seasons (summer, spring) (Howell et al., 1994).

2.3. Pathological structures

A cyst is a fluid-filled space with >25 mm in diameter in an ovary that can be >10 days in the absence of a CL (Bierschwal et al., 1975; Seguin, 1980). The infrequent presence of a cyst-like structure in conjugation with a CL is usually non-pathological. The two types of ovarian cysts are the follicular and the luteal cyst. Based on P4 concentrations or dissection of ovaries in different studies, the occurrence of luteal cysts ranges from 30% to 76% (Zemjanis, 1970; Al-Dahash and David, 1977; Dobson et al., 1977; Farin et al., 1992).

The follicular cyst presents the same ultrasonographic characteristics as a follicle, however its diameter is >25 mm and the thickness of the wall is <3 mm (Edmondson et al., 1986; Carrol et al., 1990). Its configuration is spherical, oval or polygonal depending on the relative pressure exerted by other structures on the ovary (Kahn and Leidl, 1989). The spherical shape is usually seen when there is only one cyst. Follicular cysts are anechogenic. As for the follicle, a hyperechogenic zone can be seen at the distal wall of the luteal cyst due to the presence of the luteal tissue.

Differential diagnosis between a CL with a cavity and a luteal cyst can be based on the following criteria (Kahn and Leidl, 1989):

a.) the lacuna of the CL usually has a diameter of less than 25-30 mm;

b.) the thickness of the surrounding luteal tissue ranges from 5-10 mm;

c.) the cavity of a luteal cyst is usually regular and often shows some thin white lines (trabeculae);

d.) the edge of the luteal tissue is less regular than that of a follicle (Pieterse, 1989);

e.) luteal tissue usually is wider than the cavity;

f.) the lacuna of the CL tends to regress after Day 10 of the estrous cycle (Kastelic et al., 1990a).

3. Synchronization of estrus by inducing luteolysis

The control of the estrous cycle is dependent on manipulation of the hormonal events occurring during the normal ovarian/estrous cycle. The fall in peripheral P4 concentrations may be manipulated artificially in two ways:

a.) By artificial induction of premature luteolysis using luteolytic agents, e.g. the prostaglandins.

b.) By stimulation of CL function with administration of progesterone (or one of its synthetic derivatives) for a number of days, followed by abrupt withdrawal. The effect of progestagen treatment on the ovary will be not discussed in the present review.

The most potent luteolytic agents available are derivatives of prostaglandin F2α (PGF2α).

Injection of exogenous PGF2α or one of its analogues during the mid-luteal phase of the cycle results in a premature luteolysis and consequential fall in peripheral P4 concentrations. This is followed by a rise in secretion of gonadotrophins and oestradiol-17β culminating in the pre-ovulatory surges, and eventual ovulation. The fall in P4 concentrations is rapid, invariably reaching basal levels within 30 hours of injection. There are now several

commercial analogues of PGF2α, which are all closely related to the effect of natural PGF2α (Peters and Ball, 1995).

During the past 35 years, several methods were developed to synchronize the time of estrus in dairy cattle. Synchronization with PGF2α still did not control the time of AI, because estrus detection continued to be necessary. When timed AI after PGF2α in lactating dairy cows was examined, pregnancy rates per AI was substantially lower than those for AI after a detected estrus (Archbald et al., 1992; Stevenson et al., 1987). Low pregnancy rates from timed AI using PGF2α might be partially explained by the variation in time of ovulation with respect to time of AI. Much of the variation in time to ovulation was probably due to the variation in stage of growth of the preovulatory follicle at the time of PGF2α treatment (Momont and Seguin, 1983). For example, if PGF2α was injected when a dominant follicle was fully developed and functional (i.e. Day 7 or 8 of the cycle), the time to estrus and the variation in the time to estrus were significantly less than if some dominant follicles were in the early stages of development (i.e. around Day 10 of the cycle) (Momont and Seguin, 1983; Stevenson et al., 1984).

The optimal time of AI is not well defined and probably varies among cows. The advent of successful fixed-time AI makes it important for future research to elucidate a method that allows dairy producers to select rationally an optimal time for AI (Pursley et al., 1997).

Stevens et al. (1993) reported that in Holstein-Friesian cows being on Days 8 or 10 of the estrous cycle, plasma P4 concentration decreased to <1.0 ng/ml in all cows 24 h after PGF2α treatment, similarly as reported by Edquist et al. (1974) in heifers (Swedish Red and White Breed), being on Days 8 or 14 of the estrous cycle (Table 3).

The decline in plasma P4 concentration was significantly affected (P<0,05) by the diameter of ovulatory follicle at the time of treatment. There was a linear relationship (P<0,003) between the diameter of the ovulatory follicle at cloprostenol treatment and the decline in plasma P4 concentrations 24 h after treatment. When the eventual ovulatory follicle was small (5-8 mm) at the time of cloprostenol treatment, there was a greater (P<0,05) decline in mean plasma P4 concentrations than when the ovulatory follicle was large (13-16 mm) at the time of treatment (5,8 ng/ml vs 3,9 ng/ml over 24 h) (Colazo et al., 2002). During cloprostenol-induced luteal regression, the size of the CL measured by means of ultrasonography was significantly correlated to plasma P4 concentration in heifers (Assey et al., 1993). Heifers which began estrus within 10 days after treatment showed a decrease in plasma P4 concentrations from an average of 2,5 ng/ml at treatment to 0,6 ng/ml 24 h apart. The plasma P4 concentration in

cows similarly declined from 1,9 ng/ml to 0.4 ng/ml 24 h apart (Hafs et al., 1975). Those heifers which came into heat early (1 or 2 days after treatment) had low plasma P4 concentrations (0,7±0,06 ng/ml). Thus, they were near to estrus at the time of prostaglandin treatment (Gonzalez et al., 1985).

3.1. Use of a single injection of PGF2α

3.1.1. Location of PGF2α treatment

The efficacy of PGF2α administered into the ischiorectal fossa (Colazo et al., 2002) did not differ from im application.

The intravenously (i.v.) injected PGF2α is metabolized during the first few passages through the lungs, resulting in a shorter peripheral exposure than the intramuscularly (i.m.) injected PGF2α, which is released more slowly from the injection site (Maurer, 1989). When comparing the routes of injection, a similar breeding rate, but lower conception and pregnancy rate were noted for im. vs. iv. injections. (Martineau, 2003).

When PGF2α was given either i.m. or subcutaneously (s.c.), the minimum effective dose was 20 mg for heifers and 30 mg for cows (Hafs et al., 1975). Given 500 μg cloprostenol im. or sc., the interval from treatment to estrus was significantly different (58,5±3,5 h vs. 75,0±5,4 h), but the interval to ovulation did not differ significantly (104,0±8,0 h vs. 109,3±5,8 h) (Colazo et al., 2002).

The intravulvosubmucosal administration of the prostaglandins or its analogues may reduce the dose requirement of the drug (Alvarez et al., 1991; Chatterje et al., 1989; Mishra et al., 1998).

There are numerous reports about administery reduced doses of PGF2a into various locations of the reproductive tract such as intrauterine infusion (Tervit et al., 1973; Louis et al., 1974); or injection into the uterine wall (Inskeep, 1973). Intrauterine injections can be made into the uterine horn ipsilateral to the functional CL, into the contralateral horn, or into the body of the uterus. Among cows there were no significant differences in response to treatment regardless of which uterine horn was involved (Louis et al, 1974).

Heinonen et al. (1996) concluded that intravaginal administration of 175 μg cloprostenol resulted in good estrous synchronization and pregnancy rate. Estrous synchronization was similar to that obtained with 500 μg cloprostenol administered i.m. (Table 7). Most of these approaches are much more difficult than to give an i.m. injection, and therefore are not practical for widespread use under field conditions.

3.1.2. Effect of different doses of PGF2α

When PGF2α was given either i.m. or s.c., the minimum effective dose was 20 mg for heifers and 30 mg for cows (Hafs and Manns, 1975). Stellflug et al. (1975) suggested that ovulation may occur earlier in cows after injection of 60 mg (79,0±4,5 h) than after 30 mg (90,0±5,4 h) PGF2α, but the interval to the onset of estrus (Hafs and Manns, 1975; Lagar, 1977) was not affected by the dose of PGF2α in heifers (53,8±4,3 h vs 55,8±2,0 h). Administration of either 30 mg or 20 mg PGF2α to heifers also resulted in fertility equivalent to that of control cows (30 mg=75%, 20 mg=70%, control=73%) (Roche, 1974).

Répási et al. (2003) reported that after 25 mg dinoprost the incidence of estrus and A.I. was 95%, the conception rate was 31,6%, and the average time from treatment to estrus was 3,7 day, while after 35 mg these were 84,2%, 31,2% and 2,8 day, respectively. At the same time, cows treated with 35 mg PGF2α have a shorter period from treatment to estrus and it was less variable, but the average area of luteal tissue, and the average concentration of plasma P4 on Day 0 was somewhat smaller and lower, than those in cows treated with 25 mg PGF2α. However, these differences were not statistically significant (Répási et al., 2003).

Two PG treatments 8-h apart

Archbald et al. (1993) reported that after two PGF2α (25 mg) treatments significantly (P<0,003) more cows (67% vs. 53%) showed estrus within 7 d after treatment, than those with only one treatment. In contrast, the number of prostaglandin treatments in our study (Répási et al. 2005) did not influence significantly the incidence rate of estrus. At the same time, the interval to onset of estrus was not affected by the treatment strategy (3,6±1,3 days vs. 3,6±1,2 days) in cows (Archbald et al., 1993). In agreement with these findings, the number of treatments in our study (Répási et al. 2005) also did not influence significantly the time period from treatment to oestrus.The pregnancy rate for cows treated once or twice and inseminated during the first 7 d after treatment was 28% (16/58) and 37% (27/73), respectively, but this difference did not reach a significant level (Archbald et al., 1993 and 1994). Similar conception rates (27,8%) were detected in our study, when cows were treated once. However, if the cows were treated twice (66,7%) higher conception rates were achieved, which differed significantly (P=0,0309) (Répási et al., 2005) (Table 9).

Two PG treatments 24-h apart

The percentage of cows which exhibited estrus within 7 d after treatment with two PG treatments (25 mg) 24-h apart was 57% (28/49) vs. 62% (27/47), respectively when treated

once. Pregnancy rate was 46% (treated twice) vs. 31% (treated once), respectively. The number of days from treatment to oestrus was 3,17±1,2 days (treated twice) vs. 3,7±1,11 days (treated once). Differences between the treatment protocols were not statistically significant (Archbald et al., 1993) (Table 9).

3.1.3. Effect of different drugs after intramuscular administration

Two types of prostaglandins (PGF2α) are widely used: dinoprost (a tromethamine salt (THAM) of the natural PGF2α), and cloprostenol (a synthetic analogue). Natural prostaglandin F2α has a very short half-life, once absorbed into the bloodstream; it is quickly inactivated by oxidation after one passage through the lungs (Kindahl 1980). After i.m. administration of luteolytic doses of PGF2α, plasma concentrations peaked by 10 min and declined to pre-injection values by 90 min (Stellflug et al., 1975). Cloprostenol has a longer biological half-life and is a much more potent luteolytic agent than dinoprost since it is not degraded by 15-hydroxydehydrogenase and 13,14-reductase (Bourne, 1981). According to Martinaeu (2003) the types of prostaglandins (25 mg dinoprost i.m vs. 500 μg cloprostenol i.m.) did not influence the number of cows inseminated within 7 days after treatment (85,9% vs. 82,8%), the mean day of insemination (dinoprost: 3,42 days vs. cloprostenol 3,40 days) after treatment, and the conception rate for cows (dinoprost 33,7% vs. cloprostenol 41,8%). In agreement with these findings, the number of cows with estrus and insemination (dinoprost: 50% vs. cloprostenol 50%), the time period from treatment to estrus (dinoprost: 2,88 days vs. cloprostenol 2,55 days), and the conception rate (dinoprost: 22,2 vs cloprostenol 33,3) were not differed significantly (Répási et al., 2005).

3.1.4. Average intervals to estrus

The estrus which begins during the first 2 days after prostaglandin treatment probably is initiated by normal luteolytic mechanisms before treatment, because no heifers or cows began estrus until 48 h after the second prostaglandin treatment 12 days apart (Hafs and Manns, 1975).

The above findings suggest that the considerable variation in the interval from PGF2α treatment to estrus and ovulation could be attributed to the status of the follicular wave at the time of treatment. If luteolysis is induced before the mid static phase of a dominant follicle the follicle will ovulate, resulting in a relatively short interval from treatment to ovulation, i.e. 2-3 days. If luteolysis is induced after the mid static phase of a dominant follicle, the

dominant follicle of the next wave will grow and becomes the ovulatory follicle, resulting in a longer interval from treatment to ovulation, i. e. 4-5 days (Odde, 1990; Lucy et al., 1992; Roche and Mihm, 1996). A single injection of prostaglandin F2α on Day 5 (growing phase) or Day 8 (static phase) resulted in ovulation of the dominant follicle of Wave 1, and an injection on Day 12 (regressing phase) resulted in ovulation from Wave 2 (Kastelic et al., 1990a). In heifers treated on Day 8, the dominant follicle of Wave 1 had already reached its apparent maximum diameter at the day of treatment, but its diameter increased significantly from the day of treatment to the day prior to ovulation (mean increase: 2,2 mm from the day of treatment to the day before ovulation). Subsequent preliminary observations indicated that heifers treated with PGF2α on Day 8 will ovulate from Wave 2, rather than from Wave 1 (Kastelic and Ginther, 1991). Kastelic and Ginther (1991) found the length for heifers ovulating from Wave 1: 4,2±0,1 days and Wave 2: 6,3±0,3 days, respectively. Interval to estrus after 25 mg PGF2α injection in lactating Holstein cows averaged 3,3±0,2 days (Stevenson et al., 1996). King et al. (1982) reported that this variation in time to estrus is due to the differences in the developmental stage of the preovulatory follicle at the time of PGF2α tretment and is not related to the rate of P4 decrease to basal concentrations. In crossbred beef cattle it was 54,2±4,1 hours (Twagiramungu et al., 1992). Stevenson et al. (1984) suggested that the stage of estrous cycle but not the season had a major influence on the interval to estrus in Holstein heifers after PGF2α treatment: in the early cycle (5-8 days) animals were in heat 49,5±6 h after treatment, and in late cycle (14-16 days) after 60,6±8 h, respectively.

Tanabe and Hahn (1984) examined the interval from prostaglandin treatment to estrus in three-time periods of the cycle in dairy heifers, and estrus occurred at 43,9±8,2 h (Day 7), 71,5±14,3 h (Day 11), and 53±12,2 h (Day15), respectively. In contrast, Watts and Fuquay (1985) found the duration from treatment to estrus little different: 59 h (Days 5-7), 70 h (Days 8-11), 72 h (Days 12-15), respectively. Since the PGF2α injection does not alter the dynamics of follicular growth, the time of onset of estrus is dependent on the follicular status when luteolysis is induced (Table 4).

Assey et al. (1993) found a significant negative correlation between the size of the ovulatory follicle at cloprostenol treatment and the interval to ovulation ($r=-0,56$, $P<0,05$) in dairy cattle

When the estrous response rather than the degree of synchronization was measured after PGF2α treatment, it was observed that cattle treated between Days 10-15 of the estrous cycle had a greater estrual response than cattle treated between Days 5 and 9 of the cycle (King et al., 1982; Macmillan, 1983). Other authors found the estrual response to be higher when

PGF2α was injected during the late luteal phase compared with early luteal phase (Watts and Fuquay, 1985) although some authors reported no differences (Stevenson et al., 1984; Tanabe and Hahn, 1984) (Table 5). Estrous expression was similar among seasons (Stevenson et al., 1984). With a palpable CL and treatment with 25 mg PGF2α, Archbald et al. (1994) found that the percentage of milking cows observed in estrus within 7 d after treatment was 55 % (61/111).

3.1.5. Fertility after prostaglandin treatment

Crossbred cows (n=16) were treated with 25 mg PGF2α when they had a functional CL, and were inseminated 72 or 96 h after treatment. The conception rates were 24.0% or 37.5%, respectively (Ajitkumar, 1995).

The percentages of pregnant cows after A.I. at detected estrus without treatment (control), after PGF2α treatment at a detected estrus, and at fixed timed insemination 72 h and 90 h after PGF2α treatment were 53,3%, 52,2%, and 55,8%, respectively (Lagar, 1977).

Conception rates for dairy cows inseminated twice by appointment was higher than that for cows inseminated at estrus (59% vs 40%, P<0,05) (Seguin et al., 1978). Higher pregnancy rate could be achieved when A.I. was performed after detected estrus than that after timed A.I (once). This might be partially explained by the variation in time of ovulation over periods of 5 days after PGF2α treatment (Archibald et al., 1992; Lucy et al., 1986; Stevenson et al., 1987).

It can be concluded from these studies that the pregnancy rate of cows after two fixed time inseminations was higher than that in case of one AI at fixed time, or insemination after estrus (standing heat).

Fertility following administration of dinoprost or cloprostenol was equivalent to controls when treated cows were inseminated based on observed signs of estrus (Lauderdale et al., 1974; Schultz et al., 1977). The follicular status at luteolysis does not appear to influence fertility at induced estrus. Insemination of cattle following administration of PGF2α at different stages of the estrous cycle resulted in comparable conception rates (Stevenson et al., 1984; Tanabe and Hahn, 1984), however, Watts and Fuquay (1985) found different conception rates (Table 6). Stevenson et al. (1984) did not find significant correlation between conception and estrus detection time after PGF2α. Pregnancy rates were greater in animals that ovulated the first wave dominant follicle while it was growing (GF) vs. persistent (PF) for cows 54,2% vs. 14,0% P<0,001). At estrus PF was larger than GF in cows. (Cooperative

Regional Research Project 1996). Season may suppress the luteal function, this may be a contributing factor to low fertility when cows are inseminated during summer (Howell et al., 1994). The pregnancy rate per AI was similar for cows, regardless of whether concentrations of P4 were high (>1ng/ml) or low (<1ng/ml) at the time of PGF2α treatment, but heifers with low P4 concentrations at prostaglandin treatment had a lower pregnancy rate per AI than did heifers with high P4 concentrations (Pursley et al., 1997).

Pregnancy rates after AI at an observed estrus were almost twice as great for heifers as for lactating dairy cows 71 % vs 46,3 % after the first PGF2α treatment, and 82,8% vs 45,7% after the second PGF2α injections (14 days apart) (Pursley et al., 1997), respectively.

3.2. Use of two injections and two insemination methods

The so-called 'two plus two' technique was designed to synchronize groups of animals cycling at random without prior knowledge of their precise ovarian status. All cattle are treated on Day 0 and repeated 11 (10-14) days later. Artificial insemination is then carried out at fixed time (once or twice) 3 and 4 days later or at observed oestrus.

Two injections of PG (Lutalyse) 13 days apart were given and the results were compared with not treated cows (controls). Conception rate (CR) to first AI after observed oestrus was lower in treated than in control cows (61.1% vs. 70.5%; P<0.01) (Xu et al., 1997).

Lactating Holstein-Friesian cows treated with two PGF2α 11 days apart were inseminated at 80 h (Group 1) or 72 h and 96 h (Group 2) after the second PGF2α. Conception rates were the followings: 23% (Group 1), and 30% (Group 2), control cows (untreated) (54%), respectively. Lower conception rates after timed inseminations resulted from failure of PGF2α to induce luteolysis (13% of cows) and the presence of low (<1 ng/ml) concentrations of P4 in serum (15% of cows) at the time of second injection of PGF2α. (Stevenson et al., 1987). Rosenberg et al. (1990) found that in primiparous lactating dairy cows there was a delay in the onset of estrus when PGF2α was administered at a 14-day interval compared to an 11-day interval. Conversely Selk et al. (1988) and Larson and Ball (1992) did not observe differences in time to onset of estrus in beef heifers injected with PGF2α at 11 or 14-day intervals. Lauderdale (1979) found that lactating beef cows treated with 2 PGF2α at 10-12 d apart (AI after oestrus) showed oestrus in 47% in 5 days after second treatment: first service conception rate was 61%, and pregnancy rate was 34%, respectively. While in beef heifers they were: 66%, 55%, 38%, respectively. Lactating beef cows treated with 2 PGF2α 10-12 d apart (AI at 80 h after the 2nd treatment) the pregnancy rate was 35%, and in beef heifers it was: 36%. Hafs and

Manns (1975) found no differences among the doses of PGF2α (two PGF2α inj 12 days apart). Thus, 20 mg for heifers and 30 mg for cows were sufficient to produce maximal responsiveness in ovulation control. After the second treatment (12 days apart) 88% of the dairy heifers showed oestrus in 3 to 6 days, and 68 % of the suckled beef cows. Fertility was among the heifers inseminated at 70 and 88 h after 20, 30 or 40 mg PGF2α (twice 12 days apart), 60%, 50% and 53% respectively. Among cows injected twice (12 days apart) 30 or 60 mg PGF2α 39% and 42% fertility rate was detected. Among the heifers observed in estrus during the 3rd to 6th day after the second treatment of PGF2α, the average interval to the onset of estrus was 3,1±0,4 days, and the comparable value for cows was 2,7±0,5 days (Hafs and Manns, 1975).

Crossbred cows (n=48) were treated with 2 injections of 25 mg PGF2α intramuscularly 10 days apart. The percentage of cows showing oestrus was 75%, the interval from the 2nd PG injection to onset of estrus averaged was 71.8±1.2 h (Pawshe et al., 1991).

Dairy and beef heifers were treated twice with an 11-day interval and were inseminated at 72 and 96 h after the second treatment. The pregnancy rate was 39% in both types of heifers (Macmillan et al., 1978) (Table 8).

3.3. One of the most popular method is the so-called ’1,5 method’

Cows are treated with prostaglandin and those which show estrus are inseminated. Those which have not been seen in estrus are treated again 11 days later after the first injection and may be inseminated either at fixed time or at observed estrus. This method tends to give better results than the ’two plus two’ regime. Another advantage is the reduction in cost by the decreasing the number of treatments used and the number of inseminations per cow.

N'dama cows (n=14) were given 2 intramuscular injections of a PGF2α analogue (cloprostenol) 11 days apart and were then observed continuously from 18 h after each treatment for 7 days. The percentage of cows showing standing oestrus was 85.7% and 92.9% after the 1st and 2nd treatment, respectively (Kabugaet al., 1992).

3.4. Three or more PG treatments

Pursley et al. (1997) suggested three PGF2α treatments 14 days apart, and cows were inseminated at estrus after the first and second treatment, while after the third treatment timed AI (72 to 80 h) or AI at estrus for following 3 days were performed. The estrus detection rate in the cows was similar to heifers after treatments (48,5% ; 33,3 %; 1,4% cows; 39,7%,

37,2%; 12,8% heifers), the pregnancy rates per bred after estrus: 46,3%; 45,7%; 0% cows; 71%; 82,8%; 70% heifers. Of the lactating cows that received the third PGF2α injection, almost all were bred by timed AI (timed AI: 16,7% vs AI at estrus: 1,4%), and the pregnancy rate from this AI was very low (4,3%). In contrast, more than half of the heifers that received the third PGF2α tratment were bred after detected estrus (timed AI: 10,3% vs. AI at estrus: 12,8%), and the pregnancy rate from the timed AI in heifers was 50%.

Kristula et al. (1992) indicate that weekly use of PG started 50 d postpartum, and inseminated at estrus can result in an efficient reproductive program. Cows in the set interval of prostaglandin treatment group had shorter days to first insemination (72,5% vs. 78,3%) and higher conception rates (first service: 46,9% vs. 42%), resulting in fewer days open than cows receiving a traditional veterinary reproductive protocol that relied on rectal palpation (started 50 d postpartum) to select cows for PG treatment.

4. Failure of luteolysis

Dairy heifers and cows not observed in estrus within 10 days after treatment, mainly had low (<0,5 ng/ml heifers, <0,6 ng/ml cows) P4 concentrations at the time of treatment (Hafs et al., 1975). A value of less than 0,5 ng/ml of plasma P4 (Semambo et al., 1992), or 5 ng/ml of milk P4 concentration (Smith et al., 1998) was taken as the point at which the CL was considered to be non-functional.

Some cattle had no complete luteolysis (plasma P4 did not decrease below 1,0 ng/ml 24 h after treatment) (Hafs et al., 1975). Colazo et al. (2002) reported, when treating with a lower dose (125 μg cloprostenol) of prostaglandin on Day 7 of the estrous cycle, partial luteolysis occurred in heifers and plasma P4 concentrations declined by 24 h and then recovered to pre-treatment values by 72 h after treatment.

4.1. Failure of complete luteolysis

This might occur in 10 per cent or more of cows treated with PGs. It takes the form either of complete lack of effect on P4 followed by luteal recovery usually within 24-48 hours. Causes of luteolytic failure are not clear but may be related to several factors including:

-“Treatment too early in the luteal phase

-Non-responsiveness of some corpora lutea even in the appropriate phase of the cycle

-Incorrect injection site or technique- in the case of intramuscular injections, occasionally the material may be injected accidentally into fat or ligamentus tissue

-Short half life of the exogenous prostaglandin in the animal" (Peters and Ball, 1995).

4.2. Failure of the lack of detected heat symptoms

Silent estrus can be due to management problems in detecting estrus or true silent estrus.

In practice, one of the most frequently heard complaints among managers of high yielding dairy herds, is to detect their cows in heat. The studies of Williamson et al. (1972) and King et al. (1976) indicated that up to 40% of estrous periods frequently passed undetected. In the majority of these cases (probably $\geq$90%), the animals are usually cycling normally (Williamson et al., 1972).

High milk production may be antagonistic to the expression of estrous behaviour (Harrison et al., 1990), however, there is no firm experimental evidence that high levels of milk production per se influence mounting or standing activity. Although there is some evidence that negative energy balance (NEB) during the early postpartum period may influence whether a cow is detected in heat at the beginning of the first postpartum cycle (Berghorn et al., 1998), according to others, NEB does not reduce detectability of estrus (Villa-Goddoy et al., 1990). Cows experiencing a severe NEB can produce enough estrogens to elicit an LH surge and ovulation, but not enough to cause heat, resulting in an ovulation without heat symptoms. Others suggested that the presence of suprabasal P4 levels, being released by the breakdown of fat during the period of NEB around the moment of ovulation, can seriously depress the expression of heat symptoms (Schopper and Claus, 1990).

Primary behavioural signs (mounting, standing) may be seriously depressed by the immediate environmental conditions. It is well known for example that the expression of heat seriously decreased since the overall use of concrete floors. Cows that have foot problems show less mounting activity at estrus (Van Eerdenburg et al., 1996).

Management problems may also lead to anestrus in dairy herds if there are too few observations for estrous signs per day, observations at the wrong time of the day or during the wrong phase of the daily routine, too little time spent per observation, lack of knowledge of both primary and secondary signs of estrus (Van Eerdenburg et al., 1996).

Physiological true anestrus is often seen before puberty, during pregnancy and a few weeks after calving. Some cows resume cyclic activity after a few weeks after calving and then become anestrus. True anestrus is most often seen in high-yielding dairy cattle, first-calf heifers and beef suckle cows. Most probably the cause is insufficient production or release of gonadotropins that is needed for folliculogenesis. Rectal palpation reveals ovaries that are small, flat, smooth and can have follicles up to a size of 1,5 cm. No CL can be found.

The ovary can only respond to PG if there is a functional CL. Therefore cows not undergoing activity do not respond. The proportion of cows in this state will vary from herd to herd and with the average stage post partum.

Season of the year may influence the rate of true anoestrus: It is more common in autumn and herds that are kept indoors and fed on preserved fodder (Marion and Gier, 1968; Oxenreider and Wagner, 1971)

4.3. Long follicular phases after injection

In up to 20 per cent of cows injected with PG, although luteolysis appears to occur normally, P4 concentrations remain low for an unusually long period and this may be associated with a delay in the timing of estrus and ovulation. The problem has not been reported in heifers and certainly the cycles of adult cows would appear to be less uniform than those of heifers. The absence of one or more mature follicles could cause this specific problem (i.e. extended periods of low P4), which should not be defined as long follicular phases (Peters and Ball 1995).

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Table 1. Sensitivity (Se), specificity (Sp) and predictive (+PV, –PV) values of manual palpation (MP) for predicting the presence of growing (CL1), mid-cycle (CL2 or regressing CL3) corpus luteum compared to progesterone evaluation (P) or dissection (D) of ovaries

MP vs P CL2	n	a	b	c	d	Se	Sp	+PV	–PV	References
MP vs P CL2	142	48	13	20	61	71	82	79	75	Boyd & Munro (1979)
MP vs P CL2	82	47	9	8	18	85	67	84	69	Watson & Munro (1980)
MP vs P CL2	54	25	6	9	14	74	70	81	61	Mortimer et al. (1983)
MP vs P CL2	75	57	18					76		Pathiraja et al. (1986)
MP vs P CL2	124	80	17	10	17	89	56	82	63	Ott et al. (1986)
MP vs P CL2	192	176	16					92		Kelton et al. (1988)
MP vs P CL2	252	176	9	19	48	90	84	95	72	Kelton et al. (1991)
MP vs P CL2	137	119	18					87		Archbald et al. (1992)
MP vs P CL2	68	17	2	3	46	85	96	90	94	Ribadu et al. (1994)
Total		745	108	69	204	92	65	79	75	
MP vs D CL2	13	12	1					92		Kahn & Leidl (1986)
MP vs D CL0	62			9	53				85	Pieterse et al. (1990)
MP vs D CL1	9	4	5					44		Pieterse et al. (1990)
MP vs D CL2	36	30	6					83		Pieterse et al. (1990)
MP vs D CL3	11	7	4					64		Pieterse et al. (1990)

a: presence of corpus luteum correct, b: presence of corpus luteum incorrect, c: absence of corpus luteum correct, d: absence of corpus luteum incorrect

Table 2. Sensitivity (Se), specificity (Sp) and predictive (+PV, –PV) values of the diagnosis of the absence (CL0) or the presence of growing (CL1), mid-cycle (CL2) or regressing (CL3) corpus luteum by means of ultrasonography (U) compared to dissection (D) of ovaries

	n	MHz	a	b	c	d	Se	Sp	+PV	–PV	References
U vs. D CL0	62	5			5	57				92	Pieterse et al. (1990)
U vs. D CL1	9	5	3	6					33		Pieterse et al. (1990)
U vs. D CL2	36	5	29	7					81		Pieterse et al. (1990)
U vs. DCL2	13	5	11	2					85		Kahn & Leidl (1986)
U vs. D CL2	68	7,5	19	0	1	48	95	100	100	98	Ribadu et al. (1994)
U vs. D CL3	7	5	4	3					57		Pieterse et al. (1990)

a: presence of corpus luteum correct, b: presence of corpus luteum incorrect, c: absence of corpus luteum correct, d: absence of corpus luteum incorrect

Table 3. Mean and range of peripheral plasma levels of progesterone in heifers (Swedish Red and White Breed) before and after treatment with PGF2α (Edquist et al., 1974)

Treatment		P4 level on Day of treatment.	Days after treatment with PGF2α (P4 pg/ml) 1	2	3	4
i.m.	Mean	2,2	128	145	66	76
on Day 8	Range	1,9-2,4	90-180	50-223	35-98	33-120
s.c.	Mean	1,8	205	80	44	43
on Day 8	Range	1,1-2,8	178-258	65-95	43-58	33-55
i.m.	Mean	5,2	295	122	78	163
on Day 14	Range	3,0-7,0	145-498	38-205	35-98	40-380
s.c.	Mean	4,1	418	136	153	41
on Day 14	Range	3,6-4,9	50-1200	45-248	0-533	0-105

i.m.: intramuscularly

s.c.: subcutaneously

Table 4. Average intervals to estrus after PGF2α treatment

References	Type of animal	Treatment with a single dose of PGF2α					
		Luteal phase	Early cycle	Mid cycle	Late cycle	Follicular wave 1	Follicular wave 2
		Estrus after treatment (h) or day (d) and the number of cows (n)					
Stevenson et al. (1984)	Dairy heifer		49,5±6 h 81		60,6±8 h 83		
Tanabe and Hahn (1984)	Dairy heifer		43,9±8,2 h 38	71,5±14,3 h 29	53±12,2 h 36		
Watts and Fuquay (1985)	Dairy heifer		59 h 37	70 h 87	72 h 60		
Kastelic and Ginther (1991)	Dairy heifer					4,2±0,1 d 12	6,3±0,3 d 3
Twagiramungu et al. (1992)	Beef cattle	54,2±4,1 h 34					
Stevenson et al. (1996)	Dairy cow	3,3±0,2 d 85					

Table 5. Observed estrual response (standing and silent) after prostaglandin treatment

Reference	Rate of estrual response after treatment with a single dose of PGF2α		
	Days 5-8	Days 14-16	
Stevenson et al. (1984)	84,4% n=81	83% n=83	
	Day 7	Day 11	Day 15
Tanabe and Hahn (1984)	86,0% n=38	90,0% n=29	98% n=36
	Days 5-7	Days 8-11	Days 12-15
Watts and Fuquay (1985)	43% n=37	83,6% n=87	100% n=60

Table 6. Conception rate after administration of PGF2α in different stages of the cycle

Reference	Days (d) of the cycle, and conception rates		
Stevenson et al. (1984)	5-8 d 73,7%	14-16 d 67,4%	
Tanabe and Hahn (1984)	7 d 72%	11 d 78%	15 d 78%
Watts and Fuquay (1985)	5-7 d 56,8%	8-11 d 62,1%	12-15 d 78,3%

Table 7. Effect of the route of PGF2α treatment on estrous response and onset and conception or pregnancy rate.

Reference	Dose and animal type	N	Route	Estrous response (%)	Onset of estrus (h)	CR or PR
Chatterjee et al. (1989)	25 mg PGF2α Crossbred cow	10	Intramuscular	90	93.8	
Chatterjee et al. (1989)	10 mg PGF2α Crossbred cow	10	Intravulvosubmucosal	100	79.0	
Chatterjee et al. (1989)	10 mg PGF2α Crossbred cow	10	Intrauterine deposition	90	72.8	
Alvarez et al. (1991)	500 μg clop Crossbred heifer	10	Intramuscular	80	70,5±4,2	
Alvarez et al. (1991)	250 μg clop Crossbred heifer	10	Intramuscular	90	65,3±3,8	
Alvarez et al. (1991)	250 μg clop Crossbred heifer	10	Intravulvosubmucosal	70	57,0±4,0	
Alvarez et al. (1991)	125 μg Crossbred heifer	10	Intramuscular	60	58,0±3,0	
Alvarez et al. (1991)	125 μg clop Crossbred heifer	10	Intravulvosubmucosal	60	55,7±4,4	
Heinonen (1996)	175 μg clop Ethiopian high-land Zebu	39	into the anterior vagina at a 12-day interval	61.5		66.6 (CR)
Heinonen (1996)	500-μg clop	40	into the anterior vagina at a 12-day interval	62.6		
Heinonen (1996)	500-μg clop Ethiopian high-land Zebu	33	Intramuscular at a 12-day interval	60.6		
Mishra et al. (1998)	500 μg clop Sahival cow	6	Intrmuscular	83,33	75,0±8,18	
Mishra et al. (1998)	125 μg clop Sahival cow	6	Intramuscular	33,33	76,5±3,19	
Mishra et al. (1998)	125 μg clop Sahival cow	6	Intravulvosubmucosal ipsilateral	83,33	75,0±3,23	
Mishra et al. (1998)	25 μg clop Sahival cow	6	Intravulvosubmucosal	0,0	-	
Mishra et al. (1998)	125 μg clop Sahival cow	6	Intravulvosubmucosal contralateral	33,33	76,5±3,19	
Colazo et al. (2002)	500 μg clop. Beef heifer	9	Intramuscular		58,5±3,5	
Colazo et al. (2002)	500 μg clop. Beef heifer	9	Subcutaneous		75,0±5,4	
Martineau (2003)	500-μg PGF2α Dairy cattle	199	Intravenous	81,4		46,7 (CR) 38,1 (PR)
Martineau (2003)	500-μg PGF2α Dairy cattle	203	Intramuscular	84,1		38,7 (CR) 32,5 (PR)

Table 8. Effects of two doses of PGF2α given 11-13 days apart.

Reference	Animal type and number	Treatment doses and intervals (days)	AI at observed estrus or fixed time	CR or PR
Xu et al. (1997)	Lactating dairy cows n=1039	13 days apart control	observed observed	61.1% (CR) 70.5% (CR)
Hafs and Manns (1975)	Dairy heifers n=20 n=20 n=19 Suckled beef cows n=33 n=33	20 mg (12 d apart) 30 mg (12 d apart) 40 mg (12 d apart) 30 mg (12 d apart) 60 mg (12 d apart)	70 and 88 h 70 and 88 h 70 and 88 h 70 and 88 h 70 and 88 h	60% (PR) 50% (PR) 53% (PR) 39% (PR) 42% (PR)
Macmillan et al. (1978)	Dairy heifers n=105 Beef heifers n=119	11 d apart	72 and 96 h 72 and 96 h	39% (PR) 39% (PR)
Lauderdale (1979)	Lactating beef cows n=531 beef heifers n=462 Lactating beef cow n=642 beef heifers n=469	10-12 d apart 10-12 d apart 10-12 d apart 10-12 d apart	oestrus in 5 days after 2nd inj.: 47%. 66%, 80 h 80 h	61% (CR), 34% (PR) 55% (CR), 38% (PR) 35% (PR) 36% (PR)
Stevenson et al. (1987)	Lactating Holstein Frisian cows n=119 n=57 n=59	11 d apart 11 d apart control	80 h 72 and 96 h observed	23% (CR) 30% (CR) 54% (CR)

Table 9. Effects of two doses of PGF2α apart 8 and 24 h.

	Single dose	Two doses (25 mg) 8-h apart	Two doses (25 mg) 24-h apart
Archbald et al. (1993)			
Estrus detection rate	53%	67%	
Day to estrus	3,6±1,3 d	3,6±1,2 d	
Pregnancy rate	28%	37%	
Estrus detection rate	62%		57%
Day to estrus	3,7±1,11		3,17±1,2
Pregnancy rate	31%		46%

Chapter 2

Effect of Different Doses of Prostaglandin on the Area of Corpus Luteum, and the Largest Follicle and Progesterone Concentration in Dairy Cow

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Reproduction in Domestic Animals
2003, **38,** 423-428.

Contents

Lactating dairy cows have poor reproductive efficiency because of low fertility and low rates of estrous detection. The present experiment was carried out in two dairy farms under the same conditions. Lactating dairy cows with a mature corpus luteum determined by ultrasonography and having a follicle with a diameter of ≥ 10 mm (n=49) were randomly assigned to three groups. The first group was treated with a single dose (25 mg, n=20) of exogenous prostaglandin (PGF2α), while the second group was treated with 35 mg (n=19) on Day 0, and the third group was served as untreated (n=10). Blood samples were collected daily for analyzing progesterone concentrations. In Group 1 the incidence of estrus and A.I. in 10 days after treatment was 95 % (19/20). The conception rate was 31.6 %, and the average time to estrus after treatment was 3.7 day. In Group 2 the incidence of estrus and A.I. was 84.2 % (16/19). The conception rate was 31.2%, and the average time to estrus after treatment was 2.8 day. In the untreated group only two cows (20%) showed estrus during the examined period and none of them became pregnant. In Group 2 the percentage changes relative to the corpus luteum area decreased, and the percentage changes relative to the largest follicle area increased faster, and even the oestrus started sooner than in those cows treated with 25 mg PGF2α. However, these differences between groups were not statistically significant. At the same time, the decrease in the percentage changes relative to the area of corpora lutea and to the concentrations of P4 were statistically significant in both groups.

Introduction

During the past 25 years, several methods were developed to synchronize the time of estrus in dairy cattle. Synchronization with prostaglandin F2α is successful when cows are bred of a detected estrus and because estrous detection rates and AI are more efficient than daily detection of estrus (Stevenson and Pursley 1994).

The success of estrous induction with PGF2α depends on the presence of a functional corpus luteum. Traditionally, rectal palpation (RP) of the reproductive tract is used to identify cows with a corpus luteum eligible for PGF2α treatment. However, the ability of a palpator to identify a CL and, therefore, to select a cow eligible for PGF2α treatment may not be reliable. Ott et al. (1986), summarized the findings of multiple studies comparing RP with progesterone (P4) concentrations. Overall, a 77% (Ott et al. 1986), to 79 % (Archbald et al.

1993) agreement between the diagnosis of a CL by an experienced palpator and the progesterone concentrarion was found. It was concluded that RP may be inadequate for identifying cows with a mature CL for induction of estrus by PGF2α treatment. In case of a palpable CL, Archbald et al. (1994), found that the percentage of milking cows observed in estrus within 7 days after treatment (25 mg of PGF2α) was 55 % (61/111). This management tool still does not control the time of AI, because estrous detection continues to be necessary which is required by the lower pregnancy rate after timed AI comparing with AI after detected estrus. This might be partially explained by the variation in time of ovulation over periods of 5 days with respect to time of AI (Stevenson et al. 1987).

Various attempts have been made to overcome this variability in response to PGF2α treatments. The administration of other hormons in conjunction with PGF2α, such as progesterone, oestradiol benzoate, hCG, GnRH (Deletang 1975; De Rensis and Peter 1999; Pursley et al.1996) have been attempted. There were a better degree of synchronization but the pregnancy rate was similar to that of untreated cows. Similarly two prostaglandin injections at an 8-h interval was more effective on the incidence of luteolysis than a single injection (Archbald et al. 1993). There are several studies dealing with the effect of prostaglandin on the area changes of corpus luteum and follicles in heifers (Kastellic and Ginther 1991), and on the corpus luteum size and plasma progesterone concentrations in cattle (Assey et al. 1993), and with the effect of different doses of PGF2α on fertility (Lagar 1977), however the effect of different doses of PGF2α on the area changes of the corpus luteum, the largest follicle and the P4 concentration has not been examined. The objective of our study was to compare the effect of different doses of PGF2α (0 mg, 25 mg, 35 mg) on the corpus luteum, the largest follicle, and the progesterone concentration in dairy cattle.

Materials and methods

Animals and treatments

This study was conducted in two dairy farms of Kenézlő Dózsa Agricultural Ltd. , Kenézlő, Hungary (where the average milk production was 6744, and 5839 kilograms per cow per year, respectively) between December and May. At each farm cows were housed in free stall operation. Cows were monitored for heat two times daily (a.m. and p.m.).

Primiparous and multiparous lactating dairy crossbred cows (Holstein-Friesian and Hungarian Red and Brown), having a normal sized uterus (uterus was within the pelvic inlet as described

by Szenci et al. 1995), and normal body condition score (3,0-3,5) were used in the trial. Fourty nine cows (after Day 40- post partum) with a mature corpus luteum without cavity (transrectal ultrasonography) and a follicle with a diameter of ≥ 10 mm were randomly assigned to three groups. The first group (n=20) was treated with an intramuscular single dose (25 mg) of PGF2α (Enzaprost, Sanofi Sante Nutrition Animale, Libourne, France), while the second group (n=19) was treated with 35 mg of PGF2α (Enzaprost) on Day 0, and the third group was served as an untreated group (n=10). If heat was noticed cow was inseminated once according to the farm technology in the morning by the herd managers (standing heat), or by the veterinarian, who performed the ultrasonographic examinations (silent estrus: n=4 in Group 1 and n=2 in Group 2). The semen used for A.I. was chosen by the herd managers as part of routine management of the herd. The average conception rate for the first A.I. was 37,8 % on the farms during the examinal period. All inseminated cows were examined for pregnancy by rectal palpation at Days 45 to 75 after AI.

Ultrasonographic examinations

The positions and the diameters of the corpora lutea and the largest follicles were evaluated by using a B-mode ultrasound scanner equipped with a 7,5 MHz linear-array transducer (Type 450, Pie Medical, Maastricht, The Netherlands) from Day 0 to Day 4 after treatment daily.The first ultrasonographic examinations were performed between 8 to 10 a.m. before starting the experiment and from this time on between 16 and 18 p.m. After removing the feces the transducer was inserted into the rectum. Each ovary was scanned several times in lateromedial and dorsoventral planes to determine the position and the diameters (height, width) of the corpus luteum and the largest follicle.

Blood sampling and assay procedure

Heparinized blood samples were withdrawn daily from the jugular vein, starting immediately before treatment and subsequently after each ultrasonographic examination. The plasma was removed after centrifugation (15 min at 1500 x g) and stored at -20 ^{o}C until assayed.

Concentrations of progesterone in plasma were estimated by a direct solid-phase ^{125}I RIA method in 100 μL samples in duplicate as described in detail by Ranilla et al. (1994). A value of less than 0.5 ng/ml of progesterone was taken as the point at which the corpus luteum was considered to be non-functional, as previously suggested (Semambo et al. 1992).

Statistical analysis

The areas of the corpus luteum and the largest follicles were calculated according to the following equation: Area=0,5a×0,5b×π (a= height, b=width) (Kastelic et al. 1990a). The conception rate was calculated according to the number of pregnant cows after first A.I. / the number of cows in estrous in 10 days after treatment. The area changes of the corpora lutea and the largest follicles, and the changes in P4 concentrations (Groups 1 and 2) were expressed as percentage changes relative to initial value on Day 0. The reason for this was that absolute changes showed strong dependence on the initial area, while relative changes did not. Area and P4 changes were tested for significance on each day from Day 1 to Day 4. As changes were not normally distributed, the sign test was used. To correct for multiple testing, Bonferroni correction was applied. Differences between the effect of two treatment strategies (25mg, 35mg), based on the decrease of the corpus luteum area and P4 concentrations or the increase of the largest follicle area were tested by Mann-Whitney U-test. Differences between the two treated and the control groups in case of the mean area of the corpora lutea and the largest follicles on Day 0 were tested by Kruskal-Wallis test. The significance was calculated by Fisher's exact test. The effect of interval to estrus on conception rates after A.I., was calculated by Fisher's exact test. Differences between the conception rates were evaluated by chi-square analysis. The correlation between the area of CL on Day 0 and the plasma P4 concentration was calculated by linear regression. To estimate the changes in the areas of CL and the largest follicle, and P4 concentration analysis of variance (GLM) were performed (Armitage and Berry 1994). Statistical computations were made by S-PLUS 2000.

Results

The number of cows with estrus and A.I. during the 10-day experimental period was 95 % in the first, 84,2 % in the second and 20 % in the third group, respectively. The conception rate was 31,6%, 31,2% and 0 % in Groups 1, 2 and 3, respectively.The mean area of the luteal tissue was 350,4 mm² for cows treated with 25 mg PGF2α (Group 1), 301,1 mm² for cows treated with 35 mg PGF2α (Group 2) and 340,0 mm² for utreated cows (Group 3) on Day 0. The average plasma P4 concentration was 4,07 ng/ml in Group 1 and 2,82 ng/ml in Group 2 on Day 0 (Table 1). There were no significant differences among the three groups concerning the initial area of CL and the P4 concentrations of the treated cows on Day 0. Significant

decreases in the percentage changes relative to the area of the luteal tissue on Day 0 were detected between Days 0 and 1, Days 0 and 2, Days 0 and 3 (P= 0,0007,P=0,0007,P= 0,0002) in cows treated with 25 mg PGF2α, and between Days 0 and 2, Days 0 and 3 (P=0,0009, P=0,0039) in cows treated with 35 mg PGF2α, while there were no changes in the untreated group (Table 2). The percentage changes relative to the plasma P4 concentrations (Table 3) were significantly decreased after treatment during the first 3 days in both treated groups (Group 1: P<0,001,P<0,001,P<0,002; Group 2: P<0,001, P<0,001, P=0,016). Due to limited numbers of cows on Day 4 statistical analyses were not performed.

The mean area of the largest follicle was 151,3 mm² in Group 1, 130,1 mm² in Group 2, and 105,6 mm² in Group 3 on Day 0 (Table 1), without any significant differences among the groups. Table 4 illustrates the average increase in the percentage changes relative to the area of the largest follicle on Day 0 which was greater in Group 2 than in Group 1. However, there were no statistically significant differences between the groups and within the groups during the experiment. The percentage changes relative to the area of the largest follicles in the untreated cows showed a small increase until Day 2, than it decreased, but the rate of changes was not statistically significant.

The number of cows with estrus between Days 1-3 after treatment in Group 2 was higher than in Group 1 and all of the detected estruses occurred within 4 days after treatment. However, there were no significant differences between the treated groups. There were only two cows showing heat during the experiment in the untreated group. The conception rate was 44,5 %, and 33,4% in Groups 1 and 2 having estrus within 1-3 days after treatment. Cows having estrus on ≥ Day 4 after treatment had a conception rate of 20% (Group 1) and 25% (Group 2), respectively. Overall conception rate in Groups 1 and 2 with estrus in 1-3 days after treatment was 38,1 % (8/21), and with estrus in 4-10 days after treatment was 21,4 % (3/14). However, these differences did not reach a statistically significant level. There was a positive but non-significant correlation between the mean percentage changes relative to the area of CL on Day 0 and the interval from treatment to estrus in the treated groups. At the same time the mean percentage changes relative to the area of the largest follicle in the treated groups on Day 0 and the interval from treatment to estrus showed a negative but non-significant correlation.

The area of CL on Day 0 (Groups 1 and 2) correlated significantly to plasma P4 level (P=0,0099; R^2=0,166; R=0,408), but similar significant differences could not be found between Days 1 to 4 after treatment.

Discussion

This study compared the decrease in the percentage changes relative to the area of the CL and P4 concentration, and the increase in the percentage changes relative to the area of the largest follicle in dairy cows treated with different doses of exogenous prostaglandins. Just 2 of 10 control cows with a mature CL and a follicle with a diameter of ≥10 mm showed estrus in a 10-day examined period. Rectal palpation had no stimulating effect on the mature corpora lutea during the daily ultrasonographic examinations in the untreated group. Natural prostaglandin could cause a significant decrease in the percentage changes relative to the CL area during a five day examined period as measured by real-time B-mode ultrasonography (Table 2). Similarly, a significant decrease in the percentage changes relative to the plasma P4 concentration was detected (Table 3). There were no significant differences between the two treated groups on Day 0 regarding the area of the corpus luteum and the concentration of plasma P4. In cows treated with 35 mg PGF2α, the percentage changes relative to the area of the luteal tissue and parallel with this to the plasma P4 concentrations decreased somewhat faster on the second and third day after treatment (Tables 2 and 3), than those in cows treated with 25 mg PGF2α, however these differences did not reach a significant level.

Luteal tissue area and plasma progesterone concentration are highly correlated in heifers and cows (Kastelic et al. 1990; Sprecher et al. 1989). A positive correlation was also observed between the size of corpus luteum measured by ultrasonography and the plasma P4 concentrations during cloprostenol induced luteal regression (Assey et al. 1993). Son et al. (1995), reported that the CL area was significantly correlated to milk progesterone concentration during the estrous cycle in the cows. Similar significant correlation between the plasma P4 and the area of CL was detected in our study only just before starting the experiment on Day 0. In contrast, the increase in the percentage changes relative to the area of the largest follicle was not statistically significant (Table 4).

A considerable variation in the interval after PGF2α treatment to estrus could be attributed to the status of the follicular wave at the time of treatment. It is now well established that two or three waves of follicular development occur in the majority of bovine cycles. However, a

small proportion of cycles exhibit just one or alternatively four waves per cycle (Savio et al. 1988; Sirois and Fortune 1988; Ginther et al. 1989a, b; Knopf et al. 1989). If luteolysis is induced before the mid static phase of a dominant follicle the follicle will ovulate, resulting in a relatively short interval from treatment to ovulation, i.e. 2-3 days. If luteolysis is induced after the mid static phase of a dominant follicle the dominant follicle of the next wave will grow and become an ovulatory follicle, resulting in a longer interval from treatment to ovulation, i. e. 4-5 days (Odde 1990; Lucy et al. 1992; Roche and Mihm 1996). Kastelic and Ginther (1991), reported that the period in heifers ovulating from Wave 1 is 4,2±0,1 days and Wave 2 is 6,3±0,3 days, respectively. It can be concluded from these studies that PGF2α treatment does not alter the dynamics of follicular growth, and the time of onset of estrus is dependent on the follicular status when luteolysis is induced. Assey et al. (1993), found a significant negative correlation between the size of the ovulatory follicle at cloprostenol administration and the interval to ovulation ($r=-0,56$, $P<0,05$). In contrast we were not able to find similar correlation, however our cows were selected according to a mature CL and a follicle with a diameter of ≥ 10 mm.

Stevenson et al. (1984), reported that cows in the early cycle (5-8 days) showed heat 49,5±6 h after PGF2α treatment, while cows treated in the late cycle (14-16 days) had heat 60,6±8 h later. After treatment at Days 7, 11 and 15 of the cycle, (Tanabe and Hahn 1984) dairy heifers showed heat 43,9±8,2 h, 71,5±14,3 and 53±12,2 h, respectively. Watts and Fuquay (1985), found that heifers in 5-7 days of the cycle show heat in 59 h, in 8-11 days 70 h, in 12-15 days 72 h after PGF2α treatment, respectively. On Days 1-7 of the estrous cycle the size of corpus luteum increases (Kastelic et al. 1990; Kastelic and Ginther 1991; Assey et al. 1993) between Days 8-14 the corpus luteum has a maximized size (Kastelic et al. 1990; Tanabe and Hahn 1984), and between Day 14 to ovulation the size of CL decreases (Kastelic et al. 1990).

In our experiment positive, but non-significant correlations were found between the percentage changes relative to the area of the luteal tissue and plasma P4 concentration in both treated groups on Day 0 and the interval from treatment to estrous, however the exact time of treatment regarding the state of estrus cycle was not determined. Hafs et al. (1975) and Lagar (1977), reported that the interval to onset of estrus was not affected by the dose of PGF2α either in heifers or in cows. Stellflug et al. (1973), suggested that ovulation may occur earlier in cows after injection of 60 mg than after 30 mg PGF2a. We found that cows treated with 35 mg PGF2α have a shorter period from treatment to estrus and it was less

variable, but the average area of luteal tissue, and the average concentration of plasma P4 on Day O was somewhat smaller in Group 2 treated with 35 mg PGF2α, than those in Group 1. However, these differences were not statistically significant. Further studies are needed to confirm the advantage of using higher doses of PGF2α.

The follicular status at luteolysis does not appear to influence fertility at induced estrus. Stevenson et al. (1984), inseminated heifers following administration of PGF2α at an early (Day 5-8) or later (Day 12-15) stage of the estrous cycle. The following conception rates were achieved: Days 5-8: 73,7 %, Days 14-16: 67,4% , which is comparable with Tanabe and Hahn's (1984) results. In contrast, Watts and Fuquay (1985), reported somewhat lower conception rates (56,8%) between Days 5-7. Similar results were achieved by Kastelic et al. (1990) between Days 8-11 (62,1%) and between Days 12-15 (78,3%), respectively. In contrast, lower pregnancy rates (28% and 42%) were reported by others (Archbald et al. 1993; Kristula et al. 1992) in dairy cows which are close to our findings (Group 1: 31,2 % and Group 2: 31.6%).

In conclusion the percentage changes relative to the corpus luteum area decreased, and the percentage changes relative to the largest follicle area increased faster, and even the oestrus started sooner in cows treated with 35 mg PGF2α than in those treated with 25 mg PGF2α. However, these differences between groups were not statistically significant. At the same time, the decrease in the percentage changes relative to the area of corpora lutea and to the concentrations of P4 were statistically significant in both groups.

Acknowledgments

We thank F. Deletang (Sanofi Sante Nutrition Animale, Libourne Cedex, France) for providing Enzaprost..

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Table 1. Effect of PGF2α treatment on the incidence of estrus and conception rate

Parameter	Group 1 PGF2α (25 mg)	Group 2 PGF2α (35 mg)	Group 3 Untreated
Cows (n)	20	19	10
Corpus luteum (n)*	23	22	13
Area (mm^2) of luteal tissue on Day 0 (mean± SD)	350,4±109,2	301,1±101,1	340,0±137,4
Plasma P4 (ng/ml) on Day 0 (mean± SD)	4,07±1,46	2,82±0,99	ND
Follicle (n)**	22	21	11
Area (mm^2) of the largest follicle on Day 0 (mean± SD)	151,3±76,4	130,1±43,0	105,6±32,3
Conception rate*** n/n	6/19	5/16	0/2
%	31,6	31,2	0
Estrus and A.I. in 10 days (n)	19	16	2
(%)	95	84,2	20
No estrus	1	3	8

* Some cows had two corpora lutea and both of them were involved in the calculation

**Some cows had two follicles with similar size and both of them were involved in the calculation

***Conception rate is the number of pregnancies divided by the number of cows inseminated.

ND: not determined

Table 2. Effect of PGF2α treatment on the percentage changes relative to the area of the luteal tissue on Day 0 in the cow during the first four days after treatment

Group 1					Group 2					Group 3				
CL (n)*	Cow (n)	Mean	SD	P	CL (n)*	Cow (n)	Mean	SD	P	CL (n)*	Cow (n)	Mean	SD	P
23	20	1			22	19	1			13	10	1		
21	18	0,77	0,24	0,0007	21	18	0,87	0,36	NS	13	10	1,07	0,28	NS
18	16	0,66	0,28	0,0007	14	13	0,57	0,25	0,0009	12	9	1,12	0,33	NS
12	11	0,53	0,12	0,0002	8	7	0,43	0,21	0,0039	12	9	1,16	0,34	NS
4	3	0,49	0,11	**	3	3	0,51	0,16	**	10	8	1,02	0,24	NS

* Some cows had two corpora lutea and both of them were involved in the calculatíon

**Statistical analysis was not done

NS= not significant

Table 3. Effect of PGF2α treatment on percentage changes relative to the plasma concentration (ng/ml) of progesterone (P4) on Day 0 in the cow during the first four days after treatment

Days	Cow	Group1		Cow	Group 2		Groups 1-2
	(n)	Mean	P	(n)	Mean	P	P
0.	20	1		19	1		
1.	18	0,3	<0,001	18	0,46	<0,001	0,255
2.	16	0,15	<0,001	13	0,34	<0,001	0,02
3.	11	0,12	<0,002	7	0,17	0,016	0,064
4.	3	0,12	*	3	0,12	*	*

*Statistical analysis was not done

Table 4. Effect of PGF2α treatment on percentage changes relative to the area of the largest follicles on Day 0 in the cow during the first four days after treatment

Group 1				Group 2				Group 3			
Cow (n)	F (n) *	Mean	SD	Cow (n)	F (n) *	Mean	SD	Cow (n)	F (n) *	Mean	SD
20	22	1		19	21	1		10	11	1	
18	20	1,1	0,25	18	20	1,20	0,29	10	11	1,12	0,44
16	18	1,3	0,44	13	14	1,53	0,37	9	10	1,22	0,43
11	13	1,4	0,65	7	7	1,71	0,80	9	10	1,09	0,30
3	4	1,3	0,44	3	3	1,46	0,72	8	9	1,12	0,38

*Some cows had two large follicles with similar size and both of them were involved in the calculation

F: follicle

SD: standard deviation

Table 5. Effect of the area of the luteal tissue (Day 0) on the interval from treatment to estrus

Interval from treatment to estrus	Group 1			Group 2			Total			
	Mean area of CL (mm^2)	Cow (n)*	Pregnant (n)	Mean area of CL (mm^2)	Cow (n)*	Pregnant (n)	Mean area of CL (mm^2)	Cow (n)	Pregnant (n)	Pregnant (%)
1	279,2	2	1	245,0	1	1	267,8	3	2	66,6
2	391,5	2	1	271,2	5	1	305,6	7	2	28,6
3	423,5	5	2	309,8	6	2	361,4	11	4	36,4
4	427,4	8	2	437,3	4	1	430,7	12	3	25
5										
6										
7										
8	468,9	1	0				468,9	1	0	0
9	270,9	1	0				270,9	1	0	0

* Some cows had two corpora lutea therefore the average of them was used for the evaluation

Effect of the Type and Number of Prostaglandin Treatments on Corpus Luteum, the Largest Follicle and Progesterone Concentration in Dairy Cows

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2005, **40**,1-7

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Contents

Lactating dairy cows (n=72) with a mature corpus luteum (CL) (diameter of ≥ 17 mm determined by ultrasonography and having a follicle with a diameter of ≥ 10 mm were randomly assigned to four groups. Cows were treated with cloprostenol i.m. once or twice, or with dinoprost i.m. once or twice with an 8-h apart. The ovaries of each cow were scanned daily by transrectal ultrasonography to measure the changes in the areas of corpus luteum and the largest follicle and to determine the occurrence of ovulation. Oestrus was detected twice daily. In addition, blood sample was withdrawn from each cow daily for measuring the progesterone (P4) concentrations. Significant decreases in the percentage changes relative to areas of CL and P4 concentrations or increases in the percentage changes in the area of the largest follicle on day 0 were detected in each group during the experiment**.** However, the type of the drug and the number of the treatments had no significant effect on those parameters.

Cows ovulated with or without showing oestrus (Group A) and cows having no oestrus and ovulation (Group B) were also evaluated. In contrast with the mean area of the CL and the mean concentration of P4 on Day 0, the mean area of the largest follicles between the two groups on Day 0 differed significantly. Significant decreases in the percentage changes relative to the area of the CL and P4 concentration or increases in the percentage changes relative to the area of the largest follicle during the experiment were detected in both groups however there were no group differences.

Treatment of dairy cows with two injections of prostaglandins (cloprostenol or dinoprost) at an 8-h interval resulted in more cows being observed in oestrus within 5 d after treatment and having significantly higher pregnancy rate than those treated with a single prostaglandin injection.

Introduction

During the past 25 years, several methods were developed to synchronize the time of oestrus in dairy cattle. Synchronization with PGF2α is one of the practicable methods (Stevenson and Pursley 1994). Two types of prostaglandins (PGF2α) are widely used: dinoprost (a tromethamine salt (THAM) of the natural PGF2α), and cloprostenol (a synthetic analogue). Natural prostaglandin F2α has a very short half-life, once absorbed into the bloodstream; it is quickly inactivated by oxidation after one passage through the lungs (Kindahl 1980). After i.m. administration of luteolytic doses of PGF2α, plasma concentrations peaked by 10 min

and declined to pre-injection values by 90 min (Stellflug et al. 1975). Cloprostenol has a longer biological half-life and is a much more potent luteolytic agent than dinoprost since it is not degraded by 15-hydroxydehydrogenase and 13,14-reductase (Bourne 1981).

The success of oestrous induction with PGF2α depends on the presence of a functional corpus luteum (CL). However, there is a great variation in time of oestrus/ovulation over periods of 5 days after injection of PGF2α due to the fact that the time of onset of oestrus/ovulation is mainly dependent on the follicular status when luteolysis is induced (Odde 1990; Lucy et al. 1992; Roche and Mihm 1996). This great variation can also be confirmed by higher pregnancy rate achieved when A.I. is performed after detected oestrus than that after timed A.I. (Archbald et al. 1992; Lucy et al. 1986; Stevenson et al. 1987).

Various attempts have been made to overcome this variability in response to prostaglandin treatments. The administration of other hormones in conjunction with prostaglandin, such as progesterone, oestradiol benzoate, hCG, GnRH (Deletang 1975; De Rensis and Peter 1999; Pursley et al. 1996) were attempted. There were a better degree of synchronization but the pregnancy rate was similar to that of untreated cows. Treatment with higher doses of prostaglandins was also recommended (Lagar 1977) however, in our previous study we were not able to find any benefit of administering higher doses (25 mg vs. 35 mg) of dinoprost (Répási et al. 2003). It was also reported that two prostaglandin injections at an 8-h interval were more effective in inducing luteolysis than a single injection (Archbald et al. 1993).

The objective of our study was to compare the effect of different treatment strategies (one or two doses of cloprostenol and dinoprost in an 8 h interval) on the corpus luteum, the largest follicle, the progesterone concentration, and the time of detected oestrus and/or ovulation in dairy cow.

Materials and methods

Animals and treatments

This field study was conducted in a dairy farm of Kenézlõ Dózsa Agricultural Ltd. (the average number of the cows was 540; average milk production was 6946 kg/cow/year), Kenézlõ, Hungary, between December, 2002 and May, 2003. During the examined period in the herd the mean conception rate for the first A.I. was 34,8± 9,0% and for all A.I. was 37,6± 8,2%, respectively. The cows were housed in a free stall operation and monitored for heat two times daily (a.m. and p.m.). Primiparous and multiparous crossbred cows (Holstein-Friesian and Hungarian Red and Brown), having a normal sized uterus (uterus was within the pelvic

inlet as described by Szenci et al. 1995), and with a body condition score of 3,0-3,5 were used in the experiment (using a scale 0 to 5: Wildman et al.,1982). Cows (n=72; after Day 40 pp), which had a mature corpus luteum without a cavity, with the largest diameter of ≥ 17 mm as suggested by Colazo et al. (2002) and a follicle with the largest diameter of ≥ 10 mm were randomly assigned to four groups. Cows of group I (n=18) were treated with 500 μg cloprostenol (PGF Veyx Forte, Veyx-Pharma GmbH, Schwarzenborn, Germany) i.m., while cows of group II (n=18) were treated with 2x500 μg cloprostenol with an 8-hour interval. Cows of group III (n=18) were treated with a single dose (25 mg) of dinoprost (Enzaprost, Sanofi Sante Nutrition Animale, Libourne, France) i.m., while cows of group IV (n=18) were treated with 2x25 mg of dinoprost on day 0 with an 8-hour interval. The first treatment was performed between 8 to 10 a.m. and the second one was between 4 to 6 p.m. Cows showed standing oestrus (n=29) detected by the herd manager or experienced silent oestrus (n=10) detected by the veterinarian during ultrasonographic examinations were inseminated according to the farm technology once in the morning. Silent oestrus was verified if tonic and erect uterus, stringy oestrous mucus discharge, reddening and slightly swelling of the vulva, and a Graafian follicle with ≥ 16 mm in diameter were found. If cows (n=9) ovulated without any sign of oestrus (standing or silent) or no oestrus, no ovulation was detected during the experiment (n=24) were not inseminated. The semen used for A.I. was chosen by the herd managers as part of routine management of the herd. All inseminated cows were examined for pregnancy by rectal palpation at Days 45 to 75 after A.I.

Cows detected with standing and silent oestrus (n=39) or ovulated without any sign of oestrus (n=9) (group A: n=48), and cows having no oestrus, no ovulation detected during the experiment (group B: n=24) were also compared.

Ultrasonographic examinations

The positions and the diameters of the corpora lutea and the largest follicles were evaluated by using B-mode ultrasound scanner equipped with a 7,5 MHz linear-array transducer (Type 450, Pie Medical, Maastricht, The Netherlands) between 8-10 a.m. from day 0 (before treatment) to day 4 between 16 and 18 hours after treatment. The inseminated cows were further examined until ovulation or maximum 2 days after A.I. (once daily). After removing the faeces the transducer was inserted into the rectum, and each ovary was scanned several times in lateromedial and dorsoventral planes to determine the position and the diameters

(height, width) of the corpus luteum and the largest follicle as described by Répási et al. (2003).

Blood sampling and assay procedure

Heparinized blood samples were withdrawn daily from the jugular vein, starting immediately before treatment and subsequently after each ultrasonographic examination. The blood samples were collected for maximum 2 days after AI in cows which had ovulation (with or without showing oestrus) or during the 5-day experimental period in cows which had no ovulation and oestrus. The plasma was removed after centrifugation and stored at -20 ^{o}C until assayed.

Concentrations of progesterone (P4) in plasma were estimated by a double-antibody solid phase RIA method in duplicate as described by Ranilla et al. (1994). Briefly, volumes of 0.2 ml of plasma and standards were extracted with 3 ml of petroleum ether by shaking. After centrifugation, the aqueous phase was frozen and the organic supernatant transferred in glass tubes. The solvent was removed under reduced pressure and the residue redissolved in 0.3 ml of phosphate buffer. The diluted antiserum (0.1 ml) and the tracer (progesterone11-hemisuccinate-2 [^{125}I]-iodohistamine) were added to each tube and incubation was performed for 2 h at room temperature. After this time, the bound and unbound fractions were separated by immunoprecipitation. The supernatant was removed by aspiration and the pellets counted in the LKB gamma counter. The sensitivity of the assay was less than 0.1 ng/ml. Intra- and inter-assay coefficients of variation (n=10) were 7.6% and 9.1%, respectively (Répási et al. 2003).

A value of less than 0.5 ng/ml of P4 was taken as the point, at which the corpus luteum was considered to be non-functional, as previously suggested (Semambo et al. 1992) and used by us (Répási et al. 2003).

Statistical analysis

The areas of the corpus luteum and the largest follicles were calculated according to the following equation: Area=0,5a×0,5b×π (a=height, b=width) (Kastelic et al. 1990a). The conception rate was calculated according to the number of pregnant cows after first A.I. / the number of cows in oestrus in 4 days after treatment. The changes in the areas of the corpora lutea (CL) and the largest follicles, and the changes in P4 concentrations were expressed as percentage changes relative to initial value on day 0. The reason for this was

that absolute changes showed strong dependence on the initial area, while relative changes did not.

Before treatment (day 0) comparison of groups (in terms of P4 concentration as well as the area of CL and the largest follicles) were made by two-factor ANOVA with the type of the drug and the number of treatment as factors. Treatment effects and changes in time were evaluated by repeated measures ANOVA with the type of the drug (cloprostenol /Clop/ vs dinoprost /Dinop/) and the number of treatments (1x vs 2x) as between subject factors, and time (days 1 to 4) as within subject factor. This analysis was made separately on P4 concentration, the area of CL, and the area of the largest follicle. As changes were not normally distributed, the sign test was used. Because of multiple comparisons, Bonferroni's correction was applied.

Comparison between groups A and B was made by Student *t*-tests (day 0) and repeated measures ANOVA (days 1-4). Differences between the conception rates were evaluated by Fisher's exact test. The correlation between the area of CL on day 0 and the plasma P4 concentration, as well as between the area of the largest follicles and the plasma P4 concentration was evaluated by linear regression. Statistical computations were made by S-PLUS 2000 (Armitage and Berry 1994).

Results

On day 0 the mean area of the luteal tissue was between 304,2 mm^2 to 328,7 mm^2 and the average plasma P4 concentration was between 3,61 ng/ml and 4,13 ng/ml in the four groups. In each case the initial plasma P4 concentration was > 0,8 ng/ml. There were no significant differences among the four groups concerning the initial area of the corpus luteum (P=0,783) and the P4 concentrations (P=0,877) at the beginning of the experiment. After prostaglandin treatment, significant decreases in the percentage changes relative to the area of the luteal tissue on day 0 were detected in each group (P<0,001) during the experiment. However, the type (P=0,204) and the number of treatments (P=0,327) had no significant effects on those changes in the four groups (Table 1). Similarly significant decreases in the percentage changes relative to the plasma P4 concentrations on day 0 were detected in each group (P=0,039), however, the type (P=0,641) and the number of treatments (P=0,415) did not influence them significantly (Table 2). With the exception of day 3 after treatment (P=0,181), the area of CL correlated significantly (day 0: P=0,02, day 1: P=0,05, day 2: P=0,008, day 4: P<0,001) to the plasma P4 level during the experiment.

The mean area of the largest follicle on day 0 was between 136,9 mm^2 and 157,9 mm^2, and did not differ significantly (P=0,743) among the four groups. After prostaglandin treatment significant (P<0,001) increases in the percentage changes relative to the area of the largest follicle on day 0 were detected in each group during the experiment, while the type (P=0,299) and the number of treatments (P=0,429) showed a non significant effect on them (Table 3).

As the type and the number of treatments did not influence significantly the percentage changes relative to the area of CL, the largest follicle and the concentration of P4, cows detected with standing and silent oestrus or ovulated without any sign of oestrus (group A: n=48), and cows having no oestrus, no ovulation detected during the experiment (group B: n=24) were also compared (Table 4). The mean area of the corpus luteum ($\pm$SD) on day 0 was 316,3$\pm$89,2 mm^2 in group A and 317,4$\pm$80,8 mm^2 in group B, respectively. The difference between the two groups was non-significant (P=0,959). Significant decreases (P<0,001) in the percentage changes relative to the area of the luteal tissue on day 0 during the experiment were detected in both groups, however there was no significant (P=0,074) differences between the two groups. Concerning the initial P4 (group A: 3,93$\pm$2,12 ng/ml; group B: 3,80$\pm$2,06 ng/ml) concentrations on day 0 there was also no significant differences (P=0,798) between the two groups (Table 5). The percentage changes relative to the plasma P4 concentrations on Day 0 during the experiment were significantly (P<0,001) decreased in both groups, and these changes did not differ significantly between the two groups (P=0,069). The mean area of the largest follicles of the cows in group A on day 0 (163,3$\pm$66,1 mm^2) was significantly (P=0,016) greater than that of the cows in group B (125,2$\pm$53,0 mm^2). Significant increases (P<0,001) in the percentage changes relative to the areas of the largest follicle on day 0 during the experiment were detected in both groups, however there was no significant (P=0,786) differences between the two groups (Table 6). A moderate, non-significant negative correlation between the area of the follicles and plasma P4 concentration during the experiment in group A, while in group B with the exception of day 0 a moderate, but positive, non-significant correlation was detected.

The number of cows with oestrus and A.I., the incidence of ovulations after oestrus, the number of ovulations without oestrous signs, the number of cows without oestrus and ovulation, and the conception rate and the average time from treatment to oestrus were given in Table 7. The number of the prostaglandin treatments with the exception of conception rates (P < 0,0309) did not influence significantly the incidence of ovulations after oestrus, the

number of ovulations without oestrous signs, the number of cows without oestrus and ovulation, and the average time from treatment to oestrus.

Discussion

This study compared the decrease in the percentage changes relative to the area of the corpus luteum and P4 concentration, and the increase in the percentage changes relative to the area of the largest follicle in dairy cows treated with different type of prostaglandins (dinoprost or cloprostenol), and different dosages (once or twice with an 8-h interval). Natural or synthetic prostaglandin caused a significant decrease in the percentage changes relative to the area of corpus luteum (Table 1) and the plasma P4 concentration (Table 2) in each group during the experiment.

Luteal tissue area and plasma P4 concentration were reported to be correlated highly in heifers and cows (Kastelic et al. 1990b; Sprecher et al. 1989). A positive correlation was also observed between the size of the corpus luteum measured by ultrasonography and the plasma P4 concentrations during cloprostenol-induced luteal regression (Assey et al. 1993). With the exception of day 3 values, similar significant correlations between the area of the corpus luteum and the plasma P4 concentration were observed in our study.

Archbald et al. (1993) reported that significantly (P<0,003) more cows (67% versus 53 %) showed oestrus within 7 d after treatment after two PGF2α (25 mg) treatments, than those received only one treatment. In contrast, the number and the type of prostaglandin treatments in our study did not influence significantly the incidence rate of oestrus. According to Martinaeu (2003) the types of prostaglandins (25 mg dinoprost i.m vs 500 μg cloprostenol i.m.) did not influence the number of cows inseminated within 7 days after treatment (85,9% vs 82,8%). This is in agreement with our study (55,6% vs. 52,8%), however all estruses were detected within 4 days after treatment.

A considerable variation in the interval after PGF2α treatment to oestrus could be attributed to the status of the follicular wave at the time of treatment (Odde 1990; Lucy et al.1992; Roche and Mihm 1996). It can be concluded from these studies that PGF2α treatment does not alter the dynamics of follicular growth, and the time of onset of oestrus is dependent on the follicular status when luteolysis is induced. Assey et al. (1993) found a significant negative correlation between the size of the ovulatory follicle at cloprostenol treatment and the interval to ovulation (r=-0,56, P<0,05). In contrast, we were not able to find a similar

correlation regarding the types of treatment. In addition, our selection criteria were based on a mature corpus luteum (≥ 17 mm in diameter) and a follicle with a diameter of ≥ 10 mm.

Archbald et al. (1993 and 1994) reported that the interval to onset of oestrus was not affected by the treatment strategy (once or twice) of PGF2α in the cow. The type of prostaglandin (dinoprost: 3,42 days vs. cloprostenol: 3,40 days) did not influence the mean time of AI after treatment (Martineau 2003). In agreement with these findings, the type and the number of treatments in our study also did not influence significantly the time period from treatment to oestrus.

The metoestrus CL has been shown to be non-responsive to a single injection of PGF2α or its analogues (Momont and Seguin 1982), and responsiveness increased with time, with maximal sensitivity at the end of the luteal phase. Watts and Fuquay (1985) described that the observed oestrual response rates were 43,0 %, 83,6%, and 100% when the PGF2α treatment was administered at days 5-7, days 8-11, and days 12-15 of the oestrous cycle, respectively. In our study, the mean areas of the corpora lutea on day 0 in groups A and B were not different significantly. Significant decreases in the percentage changes relative to the area of the luteal tissue and to the plasma P4 concentrations on day 0 in the case of the time were achieved however there were no group differences. The P4 concentration of cows having no oestrus, no ovulation (group B) after treatment showed a decrease on days 0 to 2, and reaching almost the pre-treatment level on days 3-4 after treatment. Colazo et al. (2002) reported similar findings, when treating cows with a lower dose (125 μg cloprostenol) of prostaglandin on day 7 of the oestrous cycle. When reduced doses of PGF2α were administered on day 7 of the cycle in heifers partial luteolysis occurred and plasma P4 concentrations declined by 24 h and then recovered to pre-treatment values by 72 h after treatment. Although the diameter of CL also decreased, but there was no recovery to pre-treatment diameter by the end of the observation period. Similar findings were found in our study. Large luteal cells (LLC) and endothelial cells in capillaries of the CL have PGF2α receptors. Small luteal cells (SLC) have LH receptors and respond to LH with increased secretion of P4 (McCracken et al. 1999; Niswender et al. 2000). The administration of PGF2α results in a decrease in the size of LLC that precedes a decrease in the number of SLC (Braden et al. 1988). Perhaps reduced doses of PGF2α affected the size of LLC and luteal capillary cells without affecting SLC (Colazo et al. 2002). The initial decline in P4 production may have been due to temporary degenerative changes in the endothelial cells of luteal

capillaries, which subsequently recovered; SLC would then be able to respond to LH with an increase in secretion of P4 (Juengel et al. 2000).

Causes of luteolytic failure (occurring in 10 per cent or more of the cows treated with prostaglandins) are not clear but may be related to several factors including: (1) non-responsiveness of some corpora lutea even in the appropriate phase of the cycle; (2) treatment too early in the luteal phase; (3) incorrect injection site or technique in the case of intramuscular injections; occasionally the material may be injected accidentally into fat or ligamentous tissue, (4) short half life of the exogenous prostaglandin in the animal as suggested by Peters and Ball (1995)**.** In our experiment, all of the cows selected for treatment had a mature CL with a diameter of ≥ 17 mm and > 0,8 ng/ml P4 concentration, therefore the above mentioned factors might not have any effect on our treatments. At the same time**,** the mean areas of the largest follicles on the day of treatment with prostaglandin were significantly smaller in cows in group B (no oestrus, no ovulation) than those in group A which might suggest that the growth rate of the largest follicles could also play an important role in the time of oestrus and ovulation. The longer persisting P4 concentration might influence the growth rate of the largest follicles and cause the failure of clinical signs of oestrus and ovulation in group B. At the same time in agreement with others (Wolfenson et al. 1994), a slightly greater growth rate was characteristics for follicles ovulated in group A during our experiment. Further studies are needed to confirm the role of follicular growth rate in the failure of oestrus and ovulation in dairy cow.

It is also assumable that the pulsatile release of uterine PGF2α and further lysis of the corpus luteum might cause the failure of oestrus and ovulation in group B. Namely, when exogenous PGF2α is administered to diestrual cows there is an immediate increase in luteal oxytocin secretion as a result of degranulation of the large luteal cells of the corpus luteum (Fields et al. 1992). Maximal level of luteal oxytocin is reached within 5 to 10 min and its secretion drops to basal values within 12 h (Wathes et al. 1984). This luteal oxytocin couples to its uterine receptors, which becomes available as a result of decreasing concentration of peripheral progesterone. Oxytocin then causes the pulsatile release of uterine PGF2α, which travels to the corpus luteum via the counter current mechanism to elicit further lysis of the corpus luteum. It would, therefore, appear that a major step in this mechanism is the further release of uterine PGF2α due to luteal oxytocin. As follicular atresia occurred in only 2 of 24 cows in group B, therefore the failure of oestrus and ovulation might be primarily connected with the failure of complete luteolysis confirmed by higher plasma P4 concentration in group B than

that in group A after prostaglandin treatment. However, this hypothesis needs further confirmation.

The conception rate for cows treated with dinoprost (25 mg i.m.) and cloprostenol (500 μg i.m.) according to Martineau (2003) was 33,7% and 41,8%, respectively. The pregnancy rate for cows treated once or twice and inseminated during the first 7 d after treatment was 28 % (16/58) and 37 % (27/73), respectively, and did not differ significantly (Archbald et al. 1993). Similar conception rates (27,8%) were detected in our study, when cows were treated once. However, if the cows were treated twice (66,7%) higher conception rates were achieved, which differed significantly (P=0,0309). Comparing the mean conception rate (first A.I.: 34,8± 9,0% and all A.I.: 37,6± 8,2%) of the examined period in the herd with the mean conception rate (48,7%) of the treated and inseminated cows, a higher conception rate was achieved by prostaglandin treatment which may confirm the benefit of this kind of treatments.

In summary, treatment of dairy cows with 2 luteolytic dosages of PGF2α or its synthetic analogue at an 8-h interval resulted in more cows (non-significantly) (18 vs. 21) being observed in oestrus within 5 d after treatment and having significantly higher conception rate (27,8% vs. 66,6%) than with 1 treatment. Further studies in progress should confirm the benefit of 2 prostaglandin treatments in a larger scale. At the same time, the type and the number of prostaglandin treatments had no effect on the incidence of ovulations after oestrus, the number of ovulations without oestrous signs, the number of cows without oestrus and ovulation, and the average time from treatment to oestrus.

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Table 1. Effect of different prostaglandin treatments on the percentage changes relative to the area of the luteal tissue on day 0 in cows during the first 4 days after treatment. Cows were examined until detected oestrus and/or ovulation, or if there was no oestrus and ovulation until day 4.

Days	Group I				Group II				Group III				Group IV			
	Cow	CL	Clop 1x		Cow	CL	Clop 2x		Cow	CL	Dinop 1x		Cow	CL	Dinop 2x	
	N	n	Mean %	SD	n	n	Mean %	SD	n	n	Mean %	SD	N	n	Mean %	SD
0	18	20*	1		18	20	1		18	18	1		18	19	1	
1	18	20	0,67	0,16	18	20	0,67	0,16	18	18	0,77	0,25	18	19	0,72	0,21
2	18	20	0,47	0,16	18	20	0,51	0,19	17	17	0,60	0,21	17	18	0,51	0,18
3	13	14	0,42	0,16	13	14	0,37	0,16	15	15	0,54	0,26	13	14	0,43	0,16
4	11	11	0,39	0,26	7	7	0,34	0,17	12	12	0,52	0,28	6	7	0,38	0,14

CL: corpus luteum.

Clop: cloprostenol; Dinop: dinoprost; SD: standard deviation.

Significant difference: time: P<0,001, type of treatment: P=0,204, number of treatment: P=0,327.

* Some cows had two corpora lutea and both of them were involved in the calculation.

Table 2. Effect of different prostaglandin treatments on the percentage changes relative to the plasma concentration (ng/ml) of progesterone (P4) on day 0 in the cow during the first 4 days after treatment. Blood samples were collected until ovulation (with or without oestrus) or maximum 2 days after A.I (without ovulation), or if there was no oestrus and ovulation until day 4.

Days	Group I			Group II			Group III			Group IV		
	Cow	Clop 1x		Cow	Clop 2x		Cow	Dinop 1x		Cow	Dinop 2x	
	N	Mean %	SD	n	Mean %	SD	n	Mean %	SD	n	Mean %	SD
0	18	1		18	1		18	1		18	1	
1	18	0,24	0,24	18	0,14	0,11	18	0,27	0,25	18	0,12	0,09
2	18	0,12	0,16	18	0,13	0,16	18	0,19	0,26	18	0,08	0,06
3	18	0,10	0,15	18	0,16	0,21	17	0,18	0,31	17	0,08	0,11
4	15	0,05	0,05	16	0,19	0,41	16	0,23	0,40	12	0,07	0,11

Clop: cloprostenol; Dinop: dinoprost; SD: standard deviation

Significant difference: time: P=0,039, type of treatment: P=0,641, number of treatment: P=0,415

Table 3. Effect of different prostaglandin treatments on the percentage changes relative to the area of the largest follicles on day 0 in the cow during the first 4 days after treatment. Cows were examined until detected oestrus and/or ovulation, or if there was no oestrus and ovulation until day 4.

Days	Group I				Group II				Group III				Group IV			
	Cow	F	Clop 1x		Cow	F	Clop 2x		Cow	F	Dinop 1x		Cow	F	Dinop 2x	
	N	n	Mean %	SD	n	n	Mean %	SD	n	n	Mean %	SD	n	n	Mean %	SD
0	18	18	1		18	18	1		18	18	1		18	19*	1	
1	18	18	1,18	0,30	18	18	1,37	0,63	18	18	1,29	0,39	18	19	1,24	0,38
2	18	18	1,46	0,50	18	18	1,35	0,45	17	17	1,42	0,56	17	18	1,27	0,35
3	15	15	1,48	0,80	16	16	1,64	0,83	16	16	1,51	0,70	12	13	1,37	0,47
4	9	9**	1,65	1,40	11	11	1,92	1,12	13	13	1,40	0,67	8	9**	1,51	0,54

F: largest follicle; Clop: cloprostenol; Dinop: dinoprost; SD: standard deviation.

*One cow had two large follicles and both of them were involved in the calculation.

**The dominant follicle in two cows (groups 1 and 4) gradually decreased in size and disappeared.

Significant difference: time: P<0,001, type of treatment: P=0,299, number of treatment: P=0,429.

Table 4. Effect of prostaglandin treatment on the corpus luteum in the cows detected with standing and silent oestrus or ovulated without any sign of oestrus (Group A), and cows having no oestrus, no ovulation detected during the experiment (Group B).

Days	Group A					Group B				
	Cow (n)	CL (n)	Mean area (mm^2)	Mean* %	SD	Cow (n)	CL (n)	Mean area (mm^2)	Mean* %	SD
0	48	52	316,3	1^{a}		24	25	317,4	1^{c}	
1	48	52	218,0	$0,69^{b}$	0,18	24	25	232,4	$0,73^{d}$	0,23
2	46	50	163,9	$0,52^{b}$	0,20	24	25	166,2	$0,52^{d}$	0,16
3	32	34	134,5	$0,43^{b}$	0,21	22	23	145,5	$0,46^{d}$	0,18
4	16	16	129,1	$0,41^{b}$	0,24	20	21	136,2	$0,43^{d}$	0,24

CL numbers may differ according to the presence of two corpora lutea (CL)

*Percentage changes relative to the area of the corpus luteum on day 0

$^{a\text{-}b,\ c\text{-}d}P<0,001$

Table 5. Effect of prostaglandin treatment on the P4 levels in the cows detected with standing and silent oestrus or ovulated without any sign of oestrus (Group A), and cows having no oestrus, no ovulation detected during the experiment (Group B).

Days	Group A				Group B			
	Cow (n)	Mean P4 (ng/ml)	Mean* %	SD	Cow (n)	Mean P4 (ng/ml)	Mean* %	SD
0	48	3,93	1^{a}		24	3,80	1^{c}	
1	48	0,63	$0,16^{b}$	0,16	24	0,97	$0,26^{d}$	0,24
2	48	0,41	$0,11^{b}$	0,14	24	0,66	$0,17^{d}$	0,22
3	46	0,34	$0,08^{b}$	0,12	24	0,83	$0,22^{d}$	0,29
4	35	0,27	$0,07^{b}$	0,15	24	0,93	$0,24^{d}$	0,42

*Percentage changes relative to the concentration of the P4 on day 0

$^{a\text{-}b,\ c\text{-}d}P<0,001$

Table 6. Effect of prostaglandin treatment on the dominant follicles in the cows detected with standing and silent oestrus or ovulated without any sign of oestrus (Group A), and cows having no oestrus, no ovulation detected during the experiment (Group B).

Days	Group A					Group B				
	Cow (n)	F (n)	Mean area (mm^2)	Mean* %	SD	Cow (n)	F (n)	Mean area (mm^2)	Mean* %	SD
0	48	49	$163,4^{A}$	1^{a}		24	24	$125,2^{B}$	1^{c}	
1	48	49	207,6	$1,27^{b}$	0,43	24	24	156,0	$1,25^{d}$	0,47
2	46	47	228,1	$1,40^{b}$	0,45	24	24	165,4	$1,32^{d}$	0,50
3	35	36	249,2	$1,53^{b}$	0,76	24	24	181,9	$1,45^{d}$	0,63
4	19	20	268,0	$1,64^{b}$	1,06	22	22	199,3	$1,59^{d}$	0,91

*Percentage changes relative to the area of the largest follicle on day 0

$^{a\text{-}b,\ c\text{-}d}P<0,001$

$^{A\text{-}B}P=0,01$

Table 7. Effect of the number of prostaglandin treatments on the incidence of oestrus and conception rate

Parameters	PGF2α 1x	PGF2α 2x	P value
	Clop or Dinop 1x	Clop or Dinop 2x	
Cows (n)	36	36	
Oestrus and insemination (n)	18	21	NS
Ovulation in 2 days after A.I. (n)	13	20	NS
Conception rate (n)*	5	14	0,0309
Ovulation but no oestrus (n)	5	4	NS
No oestrus, no ovulation (n)	13	11	NS
Average time from treatment to oestrus (day)	2,77	2,80	NS

*Conception rate is the number of pregnancies divided by the number of cows inseminated

Clop: treatment with cloprostenol (500 μg)

Dinop: treatment with dinoprost (25 mg)

NS: not significant

Chapter 4

Effect of prostaglandin treatment on the time of ovulation in dairy cow

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(In preparation)

Abstract

Primiparous and multiparous lactating dairy crossbred cows (after Day 40 post partum) with a mature corpus luteum (CL) (diameter of $\geq$ 17 mm determined by ultrasonography) and having a follicle with a diameter of $\geq$ 10 mm were treated with prostaglandin (n=80) and if they showed estrus were inseminated (Group 1: n=39). Cows (Group 2: n=41) after detected estrus were inseminated and served as control. The ovaries of each cow were scanned daily by transrectal ultrasonography from the day of detected estrus (Day –1) until ovulation, to measure the changes in the areas of corpus luteum and the largest follicle and to determine the occurrence of ovulation.

There were no significant differences between the treated and untreated cows in terms of reduction in the area of CL and of an increase in the area of the dominant follicles, however, the average area of the follicles in Group 2 was greater than in Group 1.

The highest pregnancy rate was achieved if A.I. was done on the same day as ovulation occurred in both groups (pregnancy rate in treated group was: 62,5%, in untreated group: 66,6%). In Group 1 54,5% pregnancy rates were achieved if ovulation occurred on Day 1, or 50% on Day 2 after A.I, and 53,3% and 44,4% in Group 2, respectively. The pregnancy rate for cows ovulated before A.I. in the second group was 25%. No ovulation occurred in 7 cows until Day 2 after AI and none of them became pregnant.

Introduction

Fertility in lactating dairy cows has decreased from 66% in 1951, to about 50% in 1975, and to about 28% (Kristula et al., 1992) to 42% (Archbald et al., 1993) currently. Some factors that limit fertility of lactating dairy cows include negative energy balance (Butler and Smith 1989; Sklan et al., 1994), toxic concentrations of urea and nitrogen (Ferguson et al., 1993), heat stress (Badinga et al., 1985; Lucy et al., 1986), and vitamin and mineral deficiencies (Arechiga et al., 1994). The physiological effects of these factors have been difficult to assess because physiological and management factors (competency and skill of the inseminator, proper handling of frozen semen, correct placement of semen in the uterus of the cow, and fertility of both the semen and the cow /Archbald et al., 1993/) may alter the accuracy and reliability of estrus detection as well as the pregnancy rate after AI (Pursley et al., 1996).

Normally ovulation occurs 10-12 hours after the end of the estrus or 18-26 h after LH-peak that stimulates ovulation (Arthur et al., 1996). The life-span of the ovum is relative short, and varies from about a few hours to 10 h with a maximum of 20-24 h after ovulation while the

sperm have a longer life-span (18 to 24 h with a maximum of 30-48 h) and they have to undergo capacitation before being able to fertilize the ovum (Herman et al., 1994). After this period the fertility declines progressively. The longevity of the germ cells depend on a variety of factors, therefore it is impossible to give precise figures for their lifespans.

Trimberger and Davis (1943) reported the pregnancy rate relation to the time of AI. If the AI was done earlier than 12 h before the end of the estrus the pregnancy rate was 44%, 6 to 12 hours before the end of the estrus it was 82,5%, 6 h after the end of the estrus it was 62,5%. The pregnancy rate decreased if the AI was done later to the time of estrus. After 48 h the pregnancy rate was 0%. In a recent study, pregnancy rate at Day 28 in relation to time of ovulation using 111 dairy cows was determined (Van Eerdenburg et al., 2002). When ovulation occurred before A.I. the pregnancy rate was 30%, when ovulation occurred within 24 or 48 h after A.I. the pregnancy rate was 52%, and 50%, respectively. If ovulation occurred later than 48 hours after A.I. pregnancy rate was only 15%. It was concluded from this study that the optimal time for A.I. when ovulation took place within 24 hours after A.I. (Van Eerdenburg et al., 2002).

Prostaglandin treatment may influence the interval from the onset of estrus to ovulation and the fertility rates through the period between AI and ovulation. Colazo et al. (2002) reported that after 500 μg cloprostenol i.m. treatment in two experiments (using 6 and 9 beef heifers) the mean interval from estrus to ovulation was 28 h and 45 h, respectively. However, the fertility rate has not been examined. Similarly, the length of the interval from the day of treatment to the day of post treatment ovulation was different depending on the follicular wave (Wave 1: 4,2±0,1 days; Wave 2: 6,3±0,3 days) at treatment (Kastelic and Ginther, 1991).

The purpose of this study was to detect the time of ovulation by means of daily ultrasonographic examination in prostaglandin treated and untretated dairy cows from the day of detected estrus. The pregnancy rate in relation to the time of ovulation was also evaluated.

Materials and methods

Animals and treatments

This field study was conducted in two dairy farms of Kenézlõ Agricultural Ltd., Kenézlõ, Hungary (average milk production was 6744 kg/cow/year, and 5839 kg/cow/year, respectively) between December and May. During the examined period the mean conception

rate for the first AI was 34,8± 9,0% and the mean conception rate for all AI was 37,6± 8,2%. At each farm cows were housed in free stall operation and monitored for heat two times daily (a.m. and p.m.). Primiparous and multiparous crossbred cows after Day 40 post partum (Holstein-Friesian and Hungarian Red and Brown), having a normal sized uterus (uterus was within the pelvic inlet as described by Szenci et al., 1995), and with a body condition score of 3,0-3,5 were used in the experiment (using a scale 0 to 5: Wildman et al., 1982). Cows (n=72), which had a mature corpus luteum (longitudinal diameter ≥ 17 mm) without a cavity (Colazo et al., 2002; Répási et al., 2003) and a follicle with the largest diameter of ≥ 10 mm (Répási et al., 2003) were treated with PGF2α (Enzaprost, Sanofi Sante Nutrition Animale, Libourne, France) and if they showed estrus, were inseminated (A.I.) (Goup 1: n=39). Cows in Group 2 (n=41) were untreated and inseminated after detection of estrus. Cows were inseminated once according to the farm technology in the morning by the herd managers (standing heat). The semen used for A.I. was chosen as part of routine management of the herd. All inseminated cows were examined for pregnancy by rectal palpation at Days 45 to 75 after A.I.

Ultrasonographic examinations

The day of A.I. was considered as Day 0. The positions and the diameters of the corpora lutea and the largest follicles were started to evaluate on the day of detected estrus (Day-1) by using B-mode ultrasonographic scanner equipped with a 7,5 MHz linear-array transducer (Type 450, Pie Medical, Maastricht, The Netherlands) and continued daily until ovulation. When ovulation occurred ultrasonographic examinations were stopped and the area of CL was not determined. After removing the feces the transducer was inserted into the rectum, and each ovary was scanned several times in lateromedial and dorsoventral planes to determine the position and the diameters (height, width) of the corpus luteum and the largest follicle as described previously by Répási et al. (2003).

Statistical analysis

The areas of the corpus luteum (area of the two-dimensional CL ultrasonic image) and the areas of the largest follicle (area of the two-dimensional largest follicle ultrasonic image) were calculated according to the following equation: Area=0,5a×0,5b×π (a=height, b=width) (Kastelic et al. 1990). The conception rate was calculated according to the

number of pregnant cows after first A.I. / the number of cows inseminated at detected estrus.

Changes in the measured parameters were analysed by repeated measures ANOVA procedure, the statistical model included time of sampling and groups as independent variables (Dixon, 1990).

Results

The average area of the corpus luteum in Groups 1 and 2 measured by means of ultrasonography from the day of detected estrus until ovulation, is presented in Table 1. The distribution of the undetected CL was similar in each group during the experiment, however if ovulation occurred the presence of the CL was not examined.

The mean area of the CL in ovulated cows between Days –1 and Day 1 was 132,7 mm^2, 121,9 mm^2 , and 102,5 mm^2in Group 1, 160,5 mm^2, 137,9 mm^2, and 111,8 mm^2 in Group 2, and 199,7 mm^2, 166,1 mm^2, 145,1 mm^2 in non-ovulated cows, respectively. There were no significant differences among the groups.

The changes in the average area of the largest follicles are shown in Table 2. The area of the largest follicles were somewhat greater in untreated dairy cows and in those cows which did not ovulate. However, these differences were not statistically significant. Ovulation did not occur in 6 cows treated with prostaglandin and in 1 cow in the control group.

The time of ovulation relatively to AI was estimated by means of ultrasonography, for both groups. Five different subgroups were established: ovulation occurred before A.I., on the Day of A.I., on Day 1 after A.I., on Day 2 after AI, and the number of cows with no ovulation, respectively (Table 3). The average areas of the ovulatory follicles on the day before ovulation in Group 1 (212,3 mm^2, 229,2 mm^2, 239,9 mm^2) were somewhat smaller, than those in Group 2 (227,3 mm^2, 239,0 mm^2, 276,2 mm^2), however those differences were not statistically significant (Table 3). Some cows (Group 1: n=6 /15,4%/ and Group 2: n=1 /2,4%/) did not ovulate at all during the experiment.

The pregnancy rate in Group 1 was 62,5% if ovulation occurred on the day of A.I., 55,5% if ovulation occurred on Day 1 after A.I., and 50% if ovulation occurred on Day 2 after A.I.. The overall pregnancy rate for all groups was 48,7 %.

In Group 2 pregnancy rate was 25% for cows ovulated before A.I., and it was 66,6 %, 53,3%, and 44,4%, if ovulation occurred on Days 0 to 2, respectively. Pregnancy did not occur if

cows did not ovulate within 2 days after A.I. The overall pregnancy rate for all groups were 51,2%.

The average area of the ovulatory follicle was greater, if the ovulation occurred later after AI in both groups. The average areas of the ovulatory follicles on the day before ovulation in Group 1 were higher, then in Group 2, however these differences were not statistically significant (Table 3).

Discussion

The mean area of the CL decreased gradually after detection of estrus in each group. At the same time their mean area was consistently higher in those cows which did not show ovulation (n=7) during the experiment. However, these differences were not statistically significant among the groups and within the groups.

These changes in non-treated cows were comparable to those of previous reports (Kastelic et al., 1990; Son et al., 1995). After prostaglandin induced luteolysis, the mean diameter of the CL decreased from 17,7±2 mm at the day of PGF2α treatment to 13,2±2,4 mm at the day of ovulation in heifers and from 17,0±1,7 mm to 11,4±1,6 mm in cows (Assey et al., 1993).

Large variations in the area of the CL existed in the prostaglandin treated (Group 1) and untreated cows (Group 2) during our experiment which were as follows: Group 1: Day-1: 56,5 mm^2 to 252,9 mm^2, Day 0: 62,8 mm^2 to 227,0 mm^2, Day 1: 99,0 mm^2 to 106,0 mm^2; Group 2: Day-1: 70,7 mm^2 to 298,5 mm^2, Day 0: 78,5 mm^2 to 208,9 mm^2, Day 1: 110,0 mm^2 to 113,1 mm^2, respectively.

Similarly, Assey et al. (1993) reported pronounced individual variations in plasma P4 concentrations and in CL sizes in heifers and cows. In contrast, the luteal tissue area or the size of the CL and plasma P4 concentration were reported to be highly correlated during natural luteal regression in heifers and cows (Sprecher et al. 1989; Kastelic et al., 1990) and during induced luteolysis in heifers (Assay et al., 1993) and in cows (Répási et al., 2005) while in agreement with Assey et al. (1993) similar correlation was not found in our previous study (Répási et al., 2003). Ribadu et al. (1994) emphasised that the CL may remain ultrasonographically visible without a significant reduction in size despite the plasma P4 concentration falling to basal values. According to Kastelic et al. (1990) the CL in Holstein heifers originating from the previous ovulation cannot be detected in 2% on Day –2 , 16% on Day –1, 37% on Day 0, 62% on Day 1, 76% on Day 2, 88% on Day 3, and 100% on Days 4, when Day 0 was the day of ovulation. These findings are in agreement with our results,

because 25,6% (10/39 in Groupn 1) and 24,3% (9/37 in Group 2) of the cows had no detectable CL on the day before ovulation, and 0% (Group 1) and 5,4% (Group 2) 2 days before ovulation, respectively. At the same time, no attempt was made to determine the luteal function in the cows in which no CL was found (Smith et al., 1998). The importance of these is emphasised by the fact that 5 to 30% of inseminations are carried out when the animal is not in estrus (Smith 1982; Senger et al., 1988) and would be very important for the field to have an accurate diagnostic tool at the cow side.

According to Van Eerdenburg et al. (2002) in non-treated cows the mean area of the largest follicle was 204 mm^2 on Day 0, 205 mm^2 on Day 1, and 215 mm^2 on Day 2, respectively. Similar results were detected in our study because the mean follicular area in Group 1 was: 214,5±66,7 mm^2 on Day -1, 228,9±73,1 mm^2 on Day 0 (the day of A.I.), 239,9±88,1 mm^2 on Day 1, respectively. The areas of the largest follicles in Group 1 were somewhat smaller, than in Group 2 (Day -1: 227,1±55,1 mm^2, Day 0: 254,8±62,8 mm^2, Day 1: 276,2±80,1 mm^2), however those differences between the groups and within the groups were not statistically significant. The area of the largest follicle in cows with no ovulation also did not differ significantly.

In agreement with Van Eerdenburg et al. (2002), the mean area of the ovulatory follicle on the day before ovulation was somewhat greater but not significantly, if ovulation occurred later regarding to AI (Table 3). Some of the cows in Groups 1 (n=6) and 2 (n=1) did not ovulate at all during the experiment.

Ovulation time was compared to the date of A.I. and pregnancy rate, therefore our animals were divided into 5 subgroups which were the followings: subgroup 1: ovulated before A.I., subgroup 2: ovulated on the same day as A.I., subgroup 3: ovulated 1 day after A.I., subgroup 4: Ovulated 2 days after A.I., subgroup 5: did not ovulate during the experiment. The cows treated with PGF2α seemed to have a wider spectrum when they ovulated, (16 cows ovulated on the Day of AI (Day 0), 11 cows on Day 1 and 6 cows ovulated on Day 2 after A.I.). Compared to Group 2 which ones were inseminated at detected estrus, had the highest ovulation rate on Day 0. Hafs et al. (1975) and Lauderdale et al. (1974) showed that using PGF2α results in estrus scattered over a 5-day period and that the conception rates after PGF2α treatment were higher for inseminations at observed estrus than those at fixed-time inseminations. In another study conducted by Walker et al. (1996) the mean ovulation time relative to the first mount was 27,6±5,4 h and there was no difference between spontaneous and induced estruses (PGF2α induced).

When ovulation occurred before A.I. the pregnancy rate was 30% (Van Eerdenburg et al., 2002), and 25 % in Group 2 of our study. The highest pregnancy rate was detected if ovulation occurred on the day of A.I. in each group (62,5% Group 1 vs. 66,6% Group 2). According to Van Eerdenburg et al. (2002): the pregnancy rate was 52% if the cow ovulated within 24 hours after A.I.. Fricke (2003) indicates that there is a 24-hour window in which A.I. can be conducted in relation to ovulation. In our study 54,5% and 50% Group 1 vs. 53,3% and 44,4% Group 2 pregnancy rates were achieved if ovulation occurred within 1 or 2 days after A.I, respectively.

Van Eerdenburg et al. (2002) reported that only 15% of the cows became pregnant if they ovulated more than 48 hours after insemination. No pregnancy was detected in our study if ovulation occurred 48 h after AI.

The overall conception rate was > 50% in Groups 1 and 2, but when the cows ovulated too early or too late in relation to the time of AI the conception rate was significantly lower therefore determination of optimal time for AI is of great practical importance. If ovulation does not occur within two days after AI, second AI. may be performed. Further studies are needed to evaluate the benefit of the second AI.

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Table 1. The average area (mm^2) of corpus luteum in ovulated (Groups 1 and 2) and non-ovulated cows (± SD) on Day –1 (estrus), Day 0 (A.I.), Days 1 and 2 after A.I.

Parameter	Day –1 (estrus)	Day 0 (A.I.)	Day 1	Day 2
Group 1				
Cow (n)	33	13	2	
CL (n)	31+4*	13+1*	2	
Area (mm^2)	132,7±56,3	121,9±49,6	102,5	
Min. area(mm^2)	56,5	62,8	106,0	
Max. area(mm^2)	252,9	227,0	99,0	
NDCL (n)	2	4	5	
Ovulation (n)	-	16	11	
Group 2				
Cow (n)	40	19	3	
CL (n)	33+1*	19+1*	3	
Area (mm^2)	160,5±67,1	137,9±36,7	111,8±1,63	
Min. area(mm^2)	70,7	78,5	110,0	
Max. area(mm^2)	298,5	208,9	113,1	
NDCL (n)	3	5	6	
Ovulation (n)	4	12	15	
No ovulation (Gr 1 and Gr 2)				
Cow (n)	7	6	4	2
CL (n)	7	6	4	2
Area (mm^2)	199,7±76,0	166,1±41,0	145,1±106,3	188,5
Min. area(mm^2)	78,5	138,2	71,5	117,8
Max. area(mm^2)	293,7	245	301,6	259,2
NDCL (n)	-	1	3	5

* number of cows with double corpora lutea
NDCL: no detectable corpus luteum

Table 2. The average area (mm^2) of the largest follicle in ovulated (Groups 1 and 2) and non-ovulated cows (± SD) on Day –1 (estrus), Day 0 (A.I.), Days 1 and 2 after A.I.

Parameter	Day-1	Day 0	Day 1	
Group 1*				
Cow (n)	33	17	6	
Follicle (n)	34	17	6	
Area (mm^2)	214,5±66,7	228,9±73,1	239,9±88,1	
Min. area	88,0	141,4	149,2	
Max. area	373,1	392,7	379,3	
Group 2**				
Cow (n)	36	24	9	
Follicle (n)	37	25	9	
Area (mm^2)	227,1±55,1	254,8±62,8	276,2±80,1	
Min. area	106,8	160,2	200,3	
Max. area	380,1	478,3	433,5	
No ovulation				
Cow (n)	7	7	7	7
Follicle (n)	7	7	7	7
Area (mm^2)	264,9±166,6	248,2±176,7	258,2±169,5	282,9±164,8
Min. area	129,6	94,2	129,6	150,8
Max. area	628,3	628,3	628,3	628,3

*6 cows did not ovulate during the experiment, 1 cow had two follicles (both ovulated on Day 0).

**4 cows ovulated before A.I. and 1 cow did not ovulate during the experiment, and 1 cow had two follicles (both ovulated on Day 1).

Table 3. The ovulation time and the average area (just before the ovulation) of the ovulated follicles in connection to dates of AI and pregnancy rate.

Parameter	Ovulation before AI (n)	Ovulation on Day 0 (n)	Ovulation on Day 1 (n)	Ovulation on Day 2 (n)	No ovulation until Day 2 (n)
Group 1					
Cow (n)	0	16*	11	6	6
The area of the follicle on the day before ovulation (mm^2)**		212,3±66,1	229,2±63,1	239,9±88,1	280,3±180,4
Pregnancy rate n (%)	0 (0%)	10 (62,5%)	6 (54,5%)	3 (50%)	0 (0%)
Group 2					
Cow (n)	4	12	15*	9	1
The area of the follicle on the day before ovulation (mm^2)**	ovulated	227,3±51,1	239,0±48,6	276,2±80,1	298,5
Pregnancy rate n (%)	1 (,25%)	8 (66,6%)	8 (53,3%)	4 (44,4%)	0 (0%)

* one cow had two follicles

** There were no significant difference in the average follicle areas within the groups, and between the groups. ANOVA: P>0,1.

Summary and Conclusion of the thesis

Chapter 1

The review discuss the lifespan of the CL during the estrous cycle, the luteolytic mechanisms in the bovine corpus luteum, the changes of the P4 concentration, the diagnosis of ovarian structures by means of rectal palpation and ultrasonography, when concluded that comparing rectal palpation (RP) with P4 concentrations, there was a 77% to 79 % agreement between the diagnosis of a CL by an experienced palpator and P4 concentration The detection of a CL by ultrasonography proved 96% accurate, as judged by milk P4 concentration (> 5 ng/ml). The review show synchronization techniques of estrus by inducing luteolysis with prostaglandines. Synchronization with a single injection of PGF2α still did not control the time of AI, because estrus detection continued to be necessary. When timed AI after PGF2α in lactating dairy cows was examined pregnancy rates per AI was substantially lower than those for AI after a detected estrus. Much of the variation in time to ovulation was probably due to the variation in the stage of the growth of the preovulatory follicle at the time of PGF2α treatment. The methods of using various locationas and doses for PGF2α treatments and failure of luteolysis were also discussed.

Chapter 2

Lactating dairy cows have poor reproductive efficiency because of low fertility and low rates of estrus detection. The present experiment was carried out in two dairy farms under the same conditions. Lactating dairy cows with a mature corpus luteum determined by ultrasonography and having a follicle with a diameter of ≥ 10 mm (n=49) were randomly assigned to three groups. The first group was treated with a single dose (25 mg, n=20) of exogenous prostaglandin (PGF2α), while the second group was treated with 35 mg (n=19) on Day 0, and the third group was served as untreated (n=10). Blood samples were collected daily for analyzing progesterone concentrations. In Group 1 the incidence of estrus and A.I. in 10 days after treatment was 95 % (19/20). The conception rate was 31.6 %, and the average time to estrus after treatment was 3.7 day. In Group 2 the incidence of estrus and A.I. was 84.2 % (16/19). The conception rate was 31.2%, and the average time to estrus after treatment was 2.8 day. In the untreated group only two cows (20%) showed estrus during the examined period and none of them became pregnant. In Group 2 the percentage changes relative to the corpus luteum area decreased, and the percentage changes relative to the largest follicle area

increased faster, and even the oestrus started sooner than in those cows treated with 25 mg PGF2α. However, these differences between groups were not statistically significant. At the same time, the decrease in the percentage changes relative to the area of corpora lutea and to the concentrations of P4 was statistically significant in both groups.

Chapter 3

Lactating dairy cows (n=72) with a mature corpus luteum (CL) (diameter of ≥ 17 mm determined by ultrasonography and having a follicle with a diameter of ≥ 10 mm were randomly assigned to four groups. Cows were treated with cloprostenol i.m. once or twice, or with dinoprost i.m. once or twice with an 8-h apart. The ovaries of each cow were scanned daily by transrectal ultrasonography to measure the changes in the areas of corpus luteum and the largest follicle and to determine the occurrence of ovulation. Oestrus was detected twice daily. In addition, blood samples were withdrawn from each cow daily for measuring the progesterone (P4) concentrations. Significant decreases in the percentage changes relative to areas of CL and P4 concentrations and increases in the percentage changes in the area of the largest follicle on day 0 were detected in each group during the experiment**.** However, the type of the drug and the number of the treatments had no significant effect on those parameters.

Cows ovulated with or without showing oestrus (Group A) and cows having no oestrus and ovulation (Group B) were also evaluated. In contrast with the mean area of the CL and the mean concentration of P4 on Day 0, the mean area of the largest follicles between the two groups on Day 0 differed significantly. Significant decreases in the percentage changes relative to the area of the CL and P4 concentration and increases in the percentage changes relative to the area of the largest follicle during the experiment were detected in both groups however there were no group differences.

Treatment of dairy cows with two injections of prostaglandins (cloprostenol or dinoprost) at an 8-h interval resulted in more cows being observed in oestrus within 5 d after treatment and having significantly higher pregnancy rate than those treated with a single prostaglandin injection.

Chapter 4

Primiparous and multiparous lactating dairy crossbred cows (after Day 40 post partum) with a mature corpus luteum (CL) (diameter of ≥ 17 mm determined by ultrasonography) and having a follicle with a diameter of ≥ 10 mm were treated with prostaglandin (n=80) and if they

showed estrus were inseminated (Group 1: n=39). Cows (Group 2: n=41) after detected estrus were inseminated and served as control. The ovaries of each cow were scanned daily by transrectal ultrasonography from the day of detected estrus (Day –1) until ovulation, to measure the changes in the areas of corpus luteum and the largest follicle and to determine the occurrence of ovulation.

There were no significant differences between the treated and untreated cows in terms of reduction in the area of CL and of an increase in the area of the dominant follicles, however, the average area of the follicles in Group 2 was greater than in Group 1.

The highest pregnancy rate was achieved if A.I. was done on the same day as ovulation occurred in both groups (pregnancy rate in treated group was: 62,5%, in untreated group: 66,6%). In Group 1 54,5% pregnancy rates were achieved if ovulation occurred on Day 1, or 50% on Day 2 after A.I, and 53,3% and 44,4% in Group 2, respectively. The pregnancy rate for cows ovulated before A.I. in the second group was 25%. No ovulation occurred in 7 cows until Day 2 after AI and none of them became pregnant.

Conclusions

The review discuss briefly the lifespan of the CL during the estrous cycle, the luteolytic mechanisms in the bovine corpus luteum, the changes in P4 levels during estrous cycle.

CL area is significantly correlated to milk and plasma P4 concentration, and ultrasonographic assessment of the CL is a reliable method for estimating P4 concentrations during the estrous cycle in cows.

The rewiev discusses the diagnosises of ovarian structures by means of rectal palpation and ultrasonography (follicles, corpus luteum, pathological structures). Comparing rectal palpation (RP) with P4 concentrations, there was a 77% to 79 % agreement between the diagnosis of a CL by an experienced palpator and P4 concentration. The detection of a CL by ultrasonography proved 96 per cent accurate, as judged by milk P4 concentration (> 5 ng/ml). An accuracy of 100 per cent would not be expected because it has a period of two days at the end of the cycle, when the corpus luteum remains ultrasonographically visible (without a significant reduction in size) despite the plasma P4 concentration falling to basal values. It is concluded that RP may be inadequate for identifying cows for any kind of treatment.

Synchronization techniques of estrus by inducing luteolysis with prostaglandines:

Single injection of PGF2α

During the past 35 years, several methods were developed to synchronize the time of estrus in dairy cattle. Synchronization with prostaglandin F2α still did not control the time of AI, because estrus detection continued to be necessary. When timed AI after PGF2α in lactating dairy cows was examined, pregnancy rates per AI was substantially lower than those for AI after a detected estrus. Low pregnancy rates from timed AI using PGF2α might be partially explained by the variation in time of ovulation with respect to time of AI. Much of the variation in time to ovulation was probably due to the variation in stage of growth of the preovulatory follicle at the time of PGF2α treatment.

The aim of administration on various location of PGF2α was reduce the dose requirement of the drug. Most of these approaches are much more difficult than an i.m. injection, and therefore are not practical for widespread use under field conditions.

The two injections and two insemination methods:

The so-called ’two plus two’ technique was designed to synchronize groups of animals cycling at random without prior knowledge of their precise ovarian status. All cattle are

injected on Day 0 of treatment and the injection repeated 11(10-14) days later. Artificial insemination is then carried out at fixed time (once or twice) or at observed oestrus. It can be concluded from these studies that the pregnancy rate of cows after two fixed time inseminations is higher than that in case of one AI at fixed time, or insemination after detected estrus (standing heat).

One of the most popular methods is the so-called ’1,5 method’:

Cows are injected with prostaglandin and those which show estrus are inseminated. Those which have not been seen in estrus are injected again 11 days later after the first injection and may be inseminated either at fixed time or at observed estrus. This method tends to give better results than the ’two plus two’ regime. Another advantage is the reduction in cost by the decreasing the number of treatments used and the number of inseminations per cow.

The effects of different types, doses and numbers of PGF2α treatment were examined in this thesis.

In the first part of the thesis the effects of different doses (0 mg, 25 mg vs 35 mg), different types (natural vs synthetic) and different number (once vs twice 8 h apart) of prostaglandin treatments from the day of treatment (Day 0) were evaluated.

Natural or synthetic prostaglandins, one or two treatments could cause a significant decrease in the percentage changes relative to the CL area during a five day examined period. Similarly, a significant decrease in the percentage changes relative to the plasma P4 concentration was detected.

In cows treated with 35 mg PGF2α, the percentage changes relative to the area of the luteal tissue and parallel with this to the plasma P4 concentrations decreased somewhat faster on the second and third day after treatment, than those in cows treated with 25 mg PGF2α, however these differences did not reach a significant level.

Significant correlation between the plasma P4 and the area of CL was detected in our study before starting the experiment on Day 0 (different doses of natural prostaglandin). With the exception of day 3 (after treatment) values, these significant correlations between the area of the corpus luteum and the plasma P4 concentration further existed in our study.

In contrast, the increase in the percentage changes relative to the area of the largest follicle was not statistically significant.

The number and the type of treatments and the dose of prostaglandin in our study did not influence significantly the incidence rate of estrus.

Assey et al. (1993), found a significant negative correlation between the size of the ovulatory follicle at cloprostenol treatment and the interval to ovulation ($r=-0,56$, $P<0,05$). In contrast we were not able to find similar correlation however our cows were selected according to a mature CL and a follicle with a diameter of ≥ 10 mm.

In our experiment positive, but non-significant correlations were found between the percentage changes relative to the area of the luteal tissue and the interval from treatment to estrus in both treated groups on Day 0, however the exact time of treatment regarding the state of estrous cycle was not determined.

It was found that cows treated with 35 mg PGF2α had a shorter period from treatment to estrus and it was less variable, but the average area of luteal tissue, and the average concentration of plasma P4 on Day O was somewhat smaller in cows treated with 35 mg PGF2α, than those in cows treated with 25 mg. However, these differences were not statistically significant. Further studies are needed to confirm the advantage of using higher doses of PGF2α. The type and the number of treatments in our study also did not influence significantly the time period from treatment to estrus.

The conception rate of cows treated with 25 or 35 mg PGF2α was approximate by equal, but treatment of dairy cows with 2 luteolytic dosages of PGF2α or its synthetic analogue at an 8-h interval resulted significantly higher conception rate, than with 1 treatment. Comparing the mean conception rate of the examined period in the herd with the mean conception rate of the treated and inseminated cows, a higher conception rate was achieved by prostaglandin treatment which may confirm the benefit of this kind of treatments.

The type and the number of prostaglandin treatments had no effect on the incidence of ovulations after estrus, the number of ovulations without estrous signs, the number of cows without estrus and ovulation.

In our study, the mean areas of the corpora lutea on day 0 in cows ovulated with or without showing estrus and cows having no estrus and ovulation were not different significantly. Significant decreases in the percentage changes relative to the area of the luteal tissue and to the plasma P4 concentrations on day 0 in the case of the time were achieved however there were no group differences.

At the same time**,** the mean areas of the largest follicles on the day of treatment with prostaglandin were significantly smaller in cows having no oestrus, no ovulation than those in cows ovulated with or without showing estrus which might suggest that the growth rate of the largest follicles could also play an important role in the time of estrus and ovulation.

As follicular atresia occurred in only 2 of 24 cows having no estrus, no ovulation, therefore the failure of estrus and ovulation might be primarily connected with the failure of complete luteolysis (occurring in 10 per cent or more of the cows treated with prostaglandines (Peters and Ball 1995)). The P4 concentration of cows having no estrus, no ovulation after treatment showed a decrease on days 0 to 2, and reaching almost the pre-treatment level on days 3-4 after treatment. The longer persisting P4 concentration might influence the growth rate of the largest follicles and cause the failure of clinical signs of estrus and ovulation in cows without estrus and ovulation (Group B). At the same time in agreement with others (Wolfenson et al. 1994), a slightly greater growth rate was characteristics for follicles ovulated during our experiment. Further studies are needed to confirm the role of follicular growth rate in the case of failure of estrus and ovulation in dairy cow.

Just 2 of 10 control cows with a mature CL and a follicle with a diameter of ≥10 mm showed estrus in a 10-day examined period. Rectal palpation had no stimulating effect on the mature corpora lutea during the daily ultrasonographic examinations in the untreated group.

In the second part of our examinations treated (single dose) and non treated cows were evaluated. The mean areas of the CL decreased gradually after detection of estrus in each group. At the same time their mean area was consistently higher in those cows which did not show ovulation (n=7) during the experiment. However, these differences were not statistically significant among the groups and within the groups.

Large variations in the area of the CL existed in the prostaglandin treated and untreated cows during our study were detected.

In our study 0% and 5,4% of the cows had no detectablecorpus lutum 2 days before ovulation, and 25,6% and 24,3% on the day before ovulation, respectively. At the same time, no attempt was made to determine luteal function in the cows in which no CL was found (Smith et al., 1998). The importance of these is emphasised by the fact that 5 to 30% of inseminations are carried out when the animal is not in estrus (Smith 1982; Senger et al., 1988) and would be very important for the field to have an accurate diagnostic tool at the cow side.

The areas of the largest follicles in treated cows were somewhat smaller during the experiment, than in untreated cows however those differences between the groups and within the groups were not statistically significant. The area of the largest follicle in cows with no ovulation also did not differ significantly.

The mean area of the ovulatory follicle on the day before ovulation was somewhat greater but not significantly, if ovulation occurred later regarding to AI. Some of the cows in treated (n=6) and non treated groups (n=1) did not ovulate at all during the experiment.

The cows treated with PGF2α seemed to have a wider spectrum among days when they ovulated, compared to those inseminated at detected estrus, had the highest ovulation rate on Day 0.

The overall conception rate was > 50% in cows treated or not treated, but when the cows ovulated too early or too late in relation to the time of AI the conception rate was significantly lower. No pregnancy was detected in our study if ovulation occurred 48 h after AI. Therefore determination of optimal time for AI is of great practical importance. If ovulation does not occur within two days after AI second AI might be performed. Further studies are needed to evaluate the benefit of the second AI.

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New scientific results

1. The PGF2α treatment caused a statistically significant decrease in the percentage changes relative to the area (mm^2) of the luteal tissue on Day 0, and the plasma concentration (ng/ml) of progesterone (P4) in the cow treated with different doses, different types and different numbers of prostaglandin treatments during the experiment. Similar changes were detected in cows with standing and silent estrus or ovulation without any sign of estrus, and in cows having no estrus and no ovulation.

2. The PGF2α treatment caused a statistically significant increase in the percentage changes relative to the area (mm^2) of the largest follicles on Day 0, in the cow treated with different types and different numbers of prostaglandin treatments during the experiment. Similar changes were detected in cows with standing and silent estrus or ovulation without any sign of ostrus, and in cows having no estrus, and no ovulation.

3. The mean area of the largest follicles of the cows detected with standing and silent oestrus or ovulated without any sign of estrus on Day 0 (163,3$\pm$66,1 mm^2) was significantly (P=0,016) greater than that of the cows having no estrus, and no ovulation (125,2$\pm$53,0 mm^2).

4. The area of CL on Day 0 (cows treated with different doses) correlated significantly to plasma P4 level (P=0,0099), but similar significant differences could not be found between Days 1 to 4 after treatment.
With the exception of Day 3 after treatment (P=0,181), the area of CL (cow treated with different types and different numbers) correlated significantly (Day 0: P=0,02, Day 1: P=0,05, Day 2: P=0,008, Day 4: P<0,001) to the plasma P4 level during the experiment.

5. Treatment of dairy cows with 2 luteolytic dosages of PGF2α or its synthetic analogue at an 8-h interval resulted in significantly (P=0,0309) higher conception rate (27,8% vs. 66,6%) than those, with 1 dosage.

6. Cows ovulated too early or too late in relation to the time of AI the conception rate was significantly lower. Therefore determination of optimal time for AI is of great practical importance.

Acknowledgements/Köszönetnyilvánítás

Köszönettel tartozom:
Édesanyámnak, Orosz Veronikának áldozatos szülői segítségéért, biztatásáért és támogatásáért.
Feleségemnek Dr. Répásiné Dr. Fajcsik Viktóriának és gyermekünknek, Vencelnek, akik szeretetükkel mindig új erőt adtak a munka elvégzéséhez.
Dr. Szenci Ottónak, témavezetőmnek, aki irányitotta és segitette munkámat mind a kisérletek kivitelezésénél, az eredmények értékelésénél és a dolgozatok megírásánál. A kéziratok többszöri átolvasása és tanácsai nagy segítségemre voltak a PhD munkám elkészítése során.
Dr. Reiczigel Jenőnek a statisztikai elemzéseknél nyújtott segítségéért.
Dankó Bertalannak a Kenézlő Dózsa M.G. R.t. elnökének, amiért az anyagi, és szakmai segítséget biztosította a külsős finanszírozású PhD munkámhoz.
Gyürki Attilának a Kenézlő Dózsa M.G. R.t. szarvasmarha ágazatvezetőjének, Veress Gyula és Buchalter Sándor telepvezetőknek, akik mindig készséggel biztosították számomra a vizsgálatok elvégzésének helyszínét.
Ungvári Sándor és Ungvári János felhajtóknak, akik preciz munkájukkal lehetővé tették a megfelelő létszámú csoportok kialakítását.
Valamint köszönetemet fejezem ki a Nagyállat Klinika minden dolgozójának, türelmükért és segítségükért, amit munkám elvégzése során nyújtottak.

Publication list

Répási A., Beckers JF, Sulon J, Perényi Zs, Reiczigel J and Szenci O (2003): Effect of Different Doses of Prostaglandin on the Area of Corpus Luteum, and the Largest Follicle and Progesterone Concentration in Dairy Cow**.** Reproduction in Domestic Animals. **38,** 423-428.

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