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HORMONAL METHODS FOR ESTROUS CYCLE MANIPULATION IN DAIRY COWS*

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SUMMARY: Reproduction efficiency in dairy cows is declining all over the world. Epidemiologic studies suggest that diseases have greater effect on herd fertility compared to the parameters like milk production. Immediate solution to the problem of infertility may bethe application of some method of hormonal manipulation of estrous cycle in dairy cows. Reproductive system ultrasound examination enables today's veterinarians to visualize ovarian changes during the estrous cycle, giving them a chance to intervene with exogenous hormonal inhibition and/or stimulationof temporary ovarian structures at the appropriate time. Summarizing possible hormonal methods for estrous cycle manipulation we have analyzed following protocols: prostaglandin based protocols, prostaglandin + progestagensin combination, regimes using prostaglandin + gonadotropin-releasing hormone (GnRH) and prostaglandin + GnRH+ estradiol protocols. In dairy herds where estrus detection does not represent a significant problem, prostaglandin based or Select Synch protocols are methods of choice for the hormonal manipulation of estrous cycle. However, if there are problems regarding estrus detection Ovsynch or Presynch + Ovsynch could be an effective alternative for the hormonal manipulation of estrous cycle. Furthermore, fixed time artificial insemination (TAI) could be modified to coincide with the second GnRHinjection, thus reducing the time and number of visits.

Key words: *dairy cows, estrous cycle, hormonal manipulation.*

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Decline in reproduction efficiency

Genetic selection in dairy cows has apparently created a conflict situation where reproduction performance is reduced while milk production is increasing (Lucy 2001; Royal et al., 2002).Dairy cows are usually inseminated during the maximum of lactation and pregnancy is mandatory in order to achieve optimal production. The two parameters of reproduction efficiency that could illustrate the decline in reproduction performance are first-service conception rate and number of services per conception. Both of them are worsening worldwide during the last several decades. The first service conception rate in New York State decreased from 65% in 1951 to 40% in 1996, according to data presented by Butler (1998). This process is also evident in other parts of the world like Ireland (Roche, 2000), United Kingdom (Royal et al., 2000) and Australia (Macmillan et al., 1996). Summarizing records from dairy herds in Kentucky (USA) Silvia (1998) has reported an increase in number of services per conception (SPC) from 1.62 in 1972 to 2.91 in 1996. Another basic parameter of reproduction efficiency is calving interval (CI), and maximal milk production should aim at CI of 12-13 months. Data from USA are clearly indicating prolonged CI as well as increased number of inseminations needed for successful pregnancy (figure 1).

Similar negative trends of reproduction efficiency parameters have also been detected in Serbia. Data from large dairy farms agglomeration near Belgrade indicated that days open period (DO) and number of services per conception (SPC) areincreased (176 and 2.7; DO and SPC, respectively; Stojić et al., 2011), andDO between 137-146 days, and SPC value of 2.8 have been recorded by Gvozdić et al., (2011). However, epidemiologic studies suggest that diseases have greater effect on herd fertility compared to nondisease parameters like milk production (Gröhn et al., 2000), and our results corroborated that opinion (Gvozdićet al., 2011; Gvozdić et al., 2012).

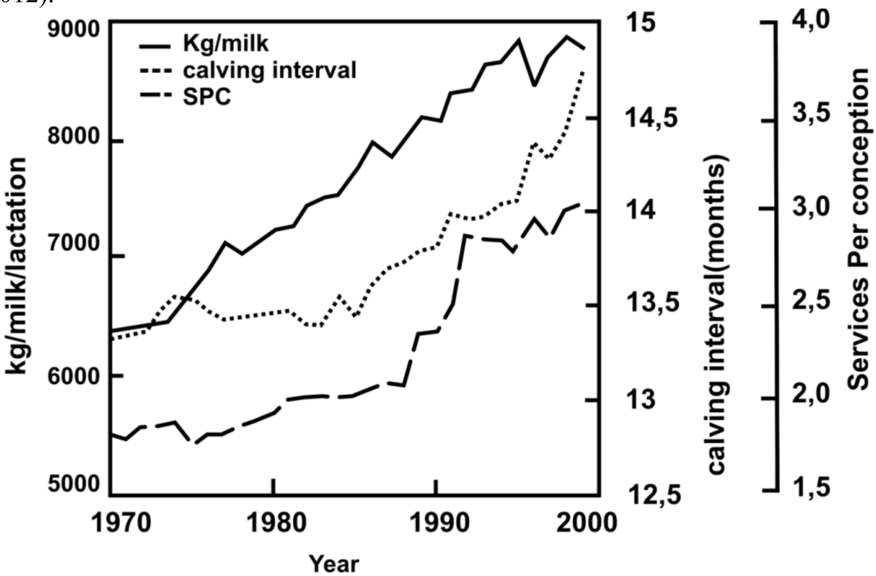

Figure 1. Milk production, calving interval and number of services per conception in dairy cows between 1970-2000 (Modified from Lucy, 2001).

In fact, higher producing herds generally have better reproductive performance (Nebel et al., 1993; Stevenson, 1999), that can probably be atributed to better feeding and reproduction management (Lucy, 2001).
When the farmers are encountered with the decline in reproduction performance an immediate solution is to use some method of hormonal intervention in order to manipulate estrous cycle in dairy cows. An ultimate goal in every case is successful pregnancy. However, there are multiple methods for estrous cycle manipulation in dairy cows, and the aim of this paper is an attempt to summarize them.

Principles of estrous cycle manipulation

Estrous cycle in dairy cows has two distinctive phases: luteal and follicular phase, with markedly different temporary endocrine structures dominating on the ovaries. Hence, there are two general principles that can be applied to the hormonal manipulation of estrus cycle in dairy cows: 1) manipulating/immitating corpus luteum life and function, and 2) manipulating follicular development and ovulation. The earliest method for estrous cycle manipulation (developed in the 1960s) was based on the use of exogenous progestagens, which means an artificial extension of the luteal phase that blocked and postponed estrus and ovulation. However, those methods weresoon abandoned since the conception rate was depressed. During the 1970s it was discovered that prostaglandin F2□ is locally active uterine hormone responsible for luteal regression and demise, and combined with progestagens it was used for estrous cycle manipulation. Despite considerable improvement there was still the problem with depressed conception rate in different hormonal methods for estrous cycle manipulation. Only after the technological breakthrough based on ultrasound during the 1980s it was discovered that persistent dominant follicle (DF) caused depressed fertility (Lucy, 2004).

Follicle development in dairy cows

The culprit for estrous cycle in dairy cows is the ovulation of dominant follicle (DF) with well manifested signs of estrus that enables successful insemination and pregnancy. In order to achieve better understanding of hormonal methods for estrous cycle manipulation here are several important facts about the development of follicles in dairy cows. A slow phase of follicular growth precedes their development in the estrous cycle and takes about 30 days. It is characterized by the increase in follicles diameter from 300 µm to 3-5 mm (Lussier et al., 1987). This period of follicular growth is independent on gonadotropin hormones. Further follicular growth is much faster (up to 1-2 mm/day) and it depends on follicle stimulating hormone (FSH) and luteinizing hormone (LH) for the final stages of development. Those changes can be detected using ultrasonography, and that could be the critical point for introducing hormonal treatment for estrous cycle manipulation.
Dairy cows usually have two to three, so called “follicular waves” during the estrous cycle. One follicular wave is composed of three phases (figure 2.): 1) **recruiting**, i.e. emerging of 4-8 follicles, less than 5 mm in diameter, 2) **selection**, when only one of the recruited follicles will continue to growth while others will undergo

atresia and demise, and 3) **dominance**, with inhibition of follicles recruitment and development, accompanied with one DF development. Only the DF from the last follicular wave during the estrous cycle will reach the final stage of development, with the possibility to ovulate and shed the oocyte. Dominant follicles from the first (and second, if there are three waves) follicular wave will undergo atresia before they reach final stage of development. Atresia of DF will initiate an increase in FSH secretion, since growing follicle secretes protein hormone inhibin that suppresses FSH secretion. The trigger of DF final development and maturation is luteal regression and a sudden decrease of progesterone blood level. The enlargement and maturation of DF with increase estradiol production will signal the preovulatory LH surge, inducing manifest estrus and ovulation.

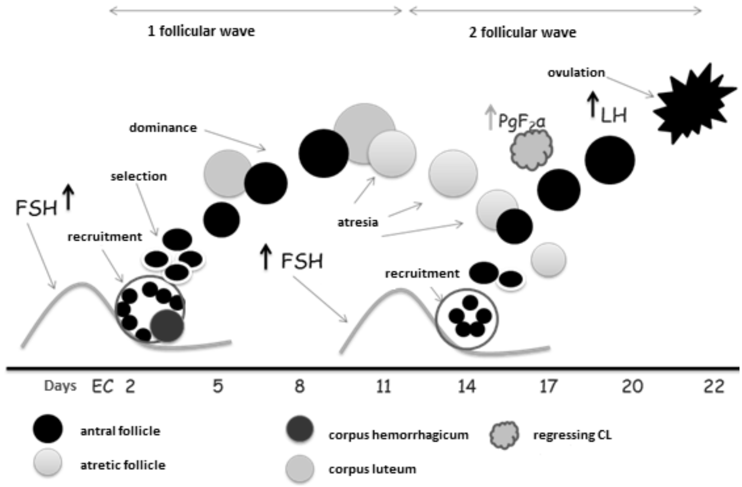

Figure 2. Follicular waves, CL development and regression (EC, estrous cycle) Modified from *Ptaszynska, 2009.*

Corpus lutem

Corpus luteum (CL) is formed after ovulation at the place of DF. The CL is possible to distinguish from the ovarian tissue as soon as two days after ovulation as an echogenic spherical or ellipsoid structure. In this phase of development CL is filled with blood coagulum (*corpus hemorrhagicum*). Function of the CL is manifested as an increase in blood progesterone level above 1 ng/mL and starts about 5 days after ovulation. Corpus luteum is increasing in size and function, to more than 25 mm CL diameter, and reaches maximal level of progesterone production at day 12.after ovulation.Luteolysis is a process of functional CL (*corpus luteumperiodicum*) decomposition that occurs between days 17-19 of estrous cycle in dairy cows, if there was no previous successful insemination. Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) secreted from the endometrium of ipsilateral uterine horn is local causative luteolytic agent. It is also the most extensively used hormone in different estrous cycle manipulation protocols.

The decrease of plasma progesterone concentration after initiation of luteolysis in nonpregnant dairy cows is faster than CL morphological changes, causing less experienced veterinarians to make mistakes regarding its function. Using measurements of blood progesterone as the indicator of CL function it was found that 25-39% of dairy cows classified as having functional CL (assessed by rectal palpation) were not producing high amounts of progesterone. Furthermore, between 15-21% of dairy cows classified as having nonfunctional CL had high blood progesterone (Senger, 2003). During ultrasound examination it is not unusual to find CL with fluid filled central cavity that is mistakenly named cystic corpus luteum. It has been proven that this finding is not pathological structure, and it could be detected as well in pregnant animals (Grygar et al., 1997; Dovenski, 1997).

$PGF_{2\alpha}$ in hormonal manipulation of estrous cycle

Natural prostaglandin ($PGF_{2\alpha}$) is effective luteolytic agent that can be used for hormonal control of estrous cycle in dairy cows. The prerequisite for successful treatment is that cow is cycling and has functional CL. It is also important to know that cow is nonpregnant,otherwise the treatment will cause embryonic mortality or abortion, since functional CL is necessary for normal pregnancy. Another disadvantage of prostaglandin based protocol is that it has no direct effect on follicular waves. Parenteral application of $PGF_{2\alpha}$ causes luteolysis, shortening luteal phase of the estrous cycle and indirectly creating favorable conditions for subsequent follicular development. Furthermore, prostaglandin treatment is effective only during specific period in the estrous cycle. This “window” in normal healthy cycling cows is opened from days 5-7 until days 17-18 after estrus. That 11-12 daysperiod represents the time during which CL is responsive to prostaglandin treatment since appropriate receptors are present. If prostaglandin treatment is issued during the first 5 days after estrus or after days 17-18 of the estrous cycle there will be no answer: at the first 5 days of estrous cycle CL is not matured enough, and after days 17-18 CL in nonpregnant dairy cows has already gone through physiologic luteolysis with no need for exogenous luteolytic agent. Hence,$PGF_{2\alpha}$treatment is usually consisted of two injections with 11 days interval in between (figure 3). If there are two follicular waves during the estrous cycle and $PGF_{2\alpha}$ is given withinthe appropriate time frame, there could be three possible outcomes: either prostaglandin injection is given early (days 7-9), at the middle (days 10-13) or late (days 14-16) in the estrous cycle. It is favorable if $PGF_{2\alpha}$ is given at the early or late days, because it is the time when DF on the ovaries can be expected, with high chance for maturation and ovulation, and 2-5 days are usually needed until the manifestation of estrus. If prostaglandin treatment is issued in the middle of the estrous cycle (days 10-13) it will take 3-7 days until estrus, since first follicular wave DF has undergone regression and atresia, while the second wave DF is underdeveloped and needs more time for maturation (Lucy, 2004).

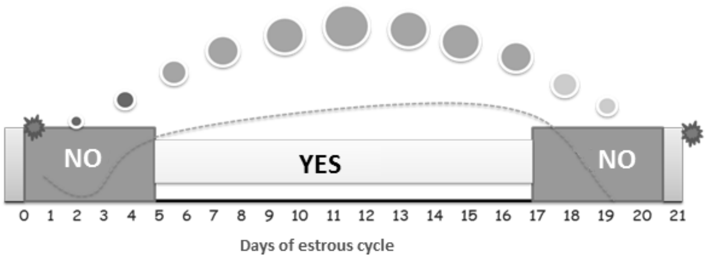

Figure 3. Prostaglandin treatment in the cycling nonpregnantdairy cows is effective during “window” between days 5-17 of the estrous cycle (marked with YES); during the first 5 days and after day 17. prostaglandin treatment is ineffective or useless (marked with NO). *Modified from Senger, 1998.*

Prostaglandin treatment alone in the cycling dairy cows requires estrus detection.Estrus signs are less pronounced in the postpartum dairy cows in negative energy balance (Westwoodet al., 2002). There is also the problem with variation of luteal phase duration in high producing dairy cows (Lucy, 2003), contributing to the limited use of prostaglandin treatment alone for hormonal manipulation of estrous cycle.

Relatively simple protocol for hormonal manipulation of the estrous cycle utilizing prostaglandin is known as “monday morning” regime (figure 4). That means prostaglandin treatment is issued for noninseminated cycling dairy cows (after voluntary waiting period, VWP) at monday morning, and estrus signs are monitored during the next 3-5 days, responders are inseminated and hopefully impregnated. If there is no estrus (no response) after the first prostaglandin treatment it is repeated 14 days later (second monday after first treatment), with estrus detection and successful insemination. The response to the first breeding treatment could be improved if one prostaglandin injection is given 14 days prior to the first insemination treatment.

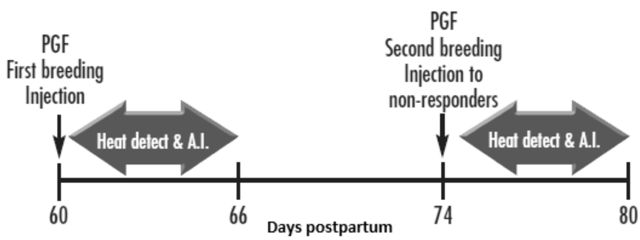

Figure 4. Monday morning protocol for hormonal manipulation of estrous cycle using PGF2a alone

Mdified from DeJarnete, 2013.

Progestagens and $PGF_{2\alpha}$ in the hormonal manipulation of estrous cycle

Hormonal manipulation of estrous cycle using progestagens is mimicking luteal phase progesterone secretion. Progesterone is important in the hormonal regulation of estrous cycle since it has a strong negative feedback on the hypothalamus, reducing the frequency of the basal episodic secretion of gonadotropin-releasing hormone (GnRH). However, the amplitude of LH pulses (together with FSH secretion), induced by the tonic GnRH secretion, is high enough to allow the development of follicles during the luteal phase. These follicles do not reach the preovulatory status until the progesterone block is removed (luteolysis). Blood progesterone concentration higher than 1 ng/mL is needed to suppress the preovulatory LH surge and estrus (Lucy, 2004). Treatment with progesterone for 14-21 days resulted in high estrus response within 3 days of progesterone removal (Macmilan et al., 1993). The negative aspect of progesterone treatment of such duration is lower conception rates, which can be overcome with shortening the time of exposure to increased progesterone level to 7 days (Lucy, 2004). Reduced fertility after long progesteron treatment originates from the fact that DF are exposed to elevated gonadotropin stimulation beyond the time when either ovulation or atresia would have occurred (Lucy, 2004, Bridges et al., 2003).Those follicles have been termed "persistent DF" and there is also negative effect on the oocyte in such follicles that could cause unsuccessful outcome of insemination (Mihm et al., 1999). Besides the reduction in duration progesterone (progestagens) treatment is usually combined with prostaglandin that removes endogenous source of progesterone and synchronize follicular waves.

There are several routes for progestagens administration: peroralltreatment (Melengesterol Acetate, MGA), intravaginal administration (Controlled Internal Drug Release, CIDR; (Progesterone-Releasing Intravaginal Device, PRID), or subcutaneous application(subcutaneous ear implants,Synchro-Mate B, Crestar method).In spite of the success in development of the MGA protocols, the use of MGA as part of any estrus synchronization protocol in beef cows represents an extralabel use of medicated feed that is prohibited in the USA by the Animal Medicinal Drug Use and Clarification Act and regulation 21 CFR 530.11(b). The feeding of MGA is specifically approved by the Food and Drug Administration (FDA, USA) regulations for estrus suppression in heifers only (0.5 mg/daily for up to 24 days).

The CIDR® (EAZI-BREED™ CIDR®, Zoetis, USA) is a T-shaped vaginal insert impregnated with natural progesterone (1.38g/insert). It is inserted intravaginally for seven days period, imitating luteal phase progesterone secretion. One day before the removal of CIDR cows are treated with the prostaglandin for the elimination of potential endogenous source of progesterone. The removal of progesterone (exogenous as well as endogenous) should create favorable conditions for the final stages of dominant follicle development and maturation. It is expected that during the next 3-5 days most of the animals exhibit estrus and can be inseminated. Furthermore, fixed time artificial insemination (TAI) at 48-64 hours after CIDR removal could be utilized. Progesterone treatment using CIDR can be used for hormonal manipulation of estrous cycle and first postpartum insemination as well as for resynchronization of nonresponsive animals (figure 5).

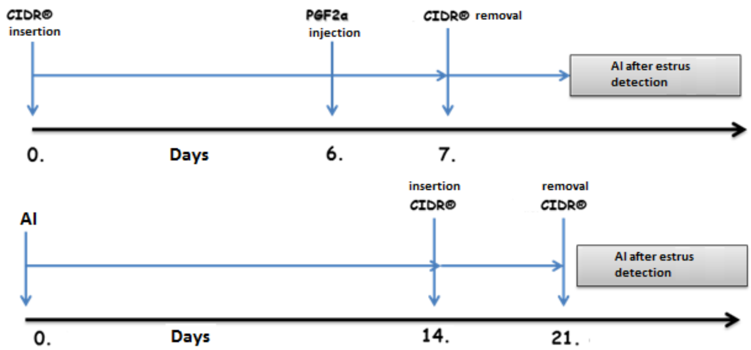

Figure 5.Progesterone treatment using CIDR for estrus synchronization and resynchronization in dairy cows. *Modified from Ptaszynska, 2006.*

The CIDR inserts have also been recommended in dairy farms where fixed time insemination protocols (TAI) were used, especially for resynchronization programs. However, the results of progesterone treatments are not unanimous: in 3 experiments inclusion of CIDR inserts to TAI protocols improved number of pregnancies/artificial insemination (P/AI) (El-Zarkouny et al., 2004, Melendez et al., 2006; Stevenson et al., 2008), whereas in 3 experiments CIDR inserts had no effect (El-Zarkouny et al., 2004, Exp. 2; Galvao et al., 2004; Stevenson et al., 2006). Furthermore, response to CIDR inserts seems to be farm dependent (Stevenson et al., 2006). Although CIDR-treated cows had greater progesterone concentrations after AI in one study (Melendez et al., 2006), the reasons why the treatment with CIDR inserts during the TAI protocol improves pregnancies per AI (P/AI) are still not clear enough. The fact that anovular cows treated with CIDR inserts were more likely to resume cyclicityin comparison with untreated cows (Gumen al., 2005; Chebel et al., 2006; Cerri et al., 2009), could indicate possible explanation, especially if we consider the fact that CIDR treatmentprovided priming with progesterone that reduced the incidence of short luteal phases after AI (Rhodes et al., 2003; Cerri et al., 2009).

GnRH and $PGF_{2\alpha}$

Hormonal manipulation of the estrous cycle using GnRH and $PGF_{2\alpha}$ in dairy cows affects both temporary ovarian endocrine structures – follicles and CL. During the follicular phase of regular estrous cycle ovulation is induced by the massive GnRH secretion from hypothalamic surge center. This mode of GnRH secretion terminates current follicular development and allows emerging of the new follicular wave. The same situation is created after exogenous GnRH analogue application, when new follicular wave starts one or two days later. When $PGF_{2\alpha}$ is applied seven days after GnRH injection all treated cows should be in the same phase of follicular development. The end point of treatment is the regression of CL induced by $PGF_{2\alpha}$ and presence of DF that finalizes its development, hopefully ovulating during the next 48-72 hours. Besides that, the GnRH induces luteinization of DF that could stimulate cyclicity in many anestrous cows (Stevenson et al., 2000). There are several variations of GnRH-$PGF_{2\alpha}$ based breeding programs commonly used in dairy herds. Each system operates from the same basic framework of GnRH and $PGF_{2\alpha}$ administered at seven-day intervals, but vary in how animals are handled for estrus detection and A.I. One of the most extensively applied combinations of hormones is known as Ovsynch, that has been developed by Pursley et al., (1995) and tested in many experimental conditions (Pursley et al., 1997; 1998; Stevenson et al., 1999). This method is also known as GPG protocol (Gonadotropin-Prostaglandin-Gonadotropin). It is based on the GnRH-$PGF_{2\alpha}$ regime, with the additional (second) GnRH injection 48 hoursafter the prostaglandin injection. The secondGnRH injection induces ovulation ofthe DF recruited afterthe first GnRH injection. No estrus detection is needed and animals areinseminated at 8-18h afterthe second GnRH(Figure 6). Cowsexpressing estrus before the scheduled TAI should beinseminated like any cow in heat anddo not need the second dose of GnRH. Despitea relatively modest results in pregnancy rate (30-40%) Ovsynch protocol has been widely used mainly because it does not require estrus detection. Furthermore, data from USA suggest that average dairy producer detects only 40% of eligible cows in estrus, with conception rate of only about 40%. Based on those data it can be calculated that in 21-day period the effective pregnancy rate is about 16% (DeJarnette, 2013). Comparing these results with 30-40% pregnancy rate from GPG protocol, with no need for estrus detection and single TAI, it is a very good effect.

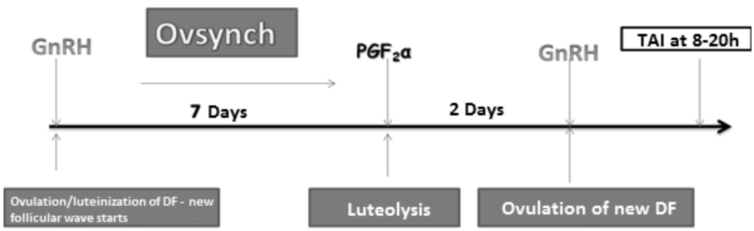

Figure 6. Ovsynch protocol (GPG method) for hormonal manipulation of estrous cycle in dairy cows (TAI – fixed time artificial insemination). *Modified from Ptaszynska, 2006.*

There are several alternative modifications to GPG protocol named: Presynch, CO-Synch, Select-Synch and Heatsynch (figure 7), and each of them should be utilized in specific situation.

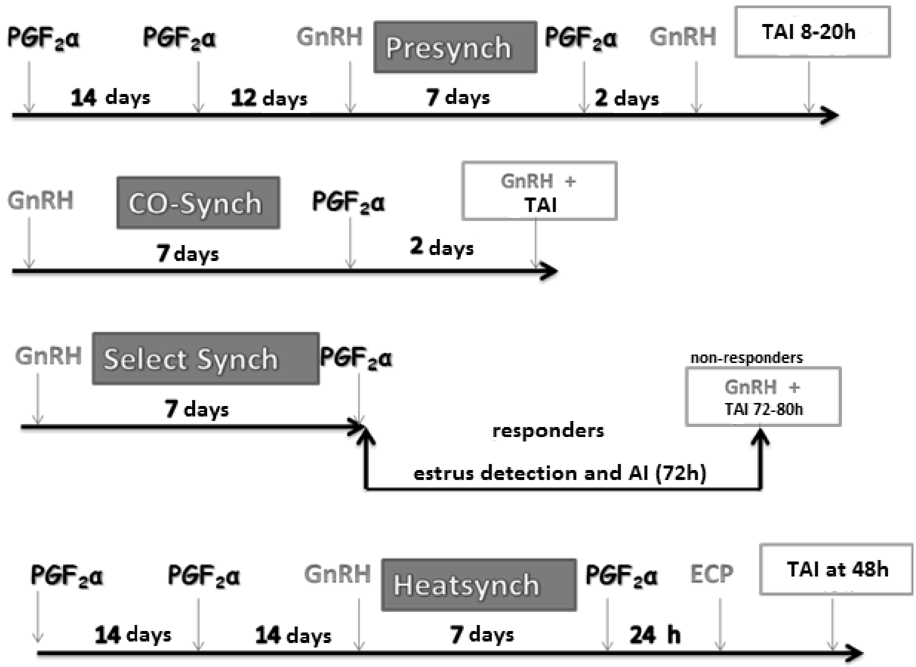

Figure 7.Alternative modifications to GPG protocol: Presynch, CO-Synch, Select-Synch and Heatsynch (ECP – estradiol cypoinate). *Modified from Ptaszynska, 2006.*

Presynch is modification of the GPG protocol that includes two prostaglandin injections with 12-14 days interval between them before the start of Ovsynch. The reasoning for such modification is based on the fact that only about 50% of cows between days 13-17 of estrous cycle have follicles able to respond to the first GnRH injection (Geary et al., 2000; Vasconcelos et al., 1999). It means that prostaglandin pretreatments actually "pre-synchronizes" cows so they will be all in the early stage of estrous cycle at the time of first GnRH injection of GPG protocol. The problem with Presynch is that it should start before the end of VWP, when it could induce luteolysis in cows having functional CL during the time of uterine involution. Then they can respond with estrus and if we inseminate them it will likely result in reduced conception rate. Therefore, introducing Presynch might actually worsen reproduction efficiency in the herd. The obvious solution to this problem is to postpone the start of Presynch until the end of VWP, which puts us in dilemma (DeJarnette, 2013): are we introducing Presynch or we implement prostaglandin based method for hormonal manipulation of estrous cycle with Ovsynch for all cows not detected in estrus? The solution is not a simple one, since Presynch can improve pregnancy rate after Ovsynch (Moreira et al., 2000; El-Zakouny et al., 2004). If we accept possible drawbacks of Presynch early start we can count on improved pregnancy rate after Ovsynchthat should be satisfactory.Possible answer to this dilemma might be the CO-Synch protocol, were the cows are originally inseminated at the time of second GnRH injection (figure 7). Modifying CO-Synch protocol with Presynch treatment starting at 32-37 days postpartum, and timing the AI and second GnRH injection at 72 hours after the last prostaglandin treatment Portaluppi et al., (2005) achieved pregnancy and calving rate of 31.4% and 29.3%, respectively.

At dairy farms where estrus detection is not a problem and AI is conducted in standing estrus Select-Synch is a method of choice for hormonal manipulation of estrous cycle. Cows responding to the treatment are detected in estrus during 3 days after prostaglandin injection and inseminated. Nonresponding cows are inseminated at fixed time between 72-80 hours after prostaglandin treatment with concurrent GnRH injection(DeJarnet et al., 2001). Using this protocol for hormonal manipulation of estrous cycle it is possible to inseminate most of the cows in standing estrus (50-70%), while the remaining animals are given the possibility to achieve pregnancy after fixed time artificial insemination. It is obvious that this protocol can save money because the second GnRH injection is used only for nonresponding dairy cows.

Major difference between Heatsynch and other protocols is in the use of estradiol cypionate at 24h after prostaglandin injection. Estradiol and its derivatives like estradiol-cypionate in this context is used instead of GnRH since it can cause increase of endogenous GnRH secretion that can induce LH surge and DF ovulation. In 75% of estradiol-treated cows submitted to the Heatsynch protocol ovulation occurred between 48-72h after ECP injection. This protocol is an alternative to GPG since combined with Presynch is comparable to Ovsynch regarding pregnancy rate (Pancarci et al., 2002).

An extensive meta-analysis of many different estrous cycle manipulation protocols (71 treatments and control comparisons) conducted by Rabiee et al., (2005) led to the conclusion that conception and pregnancy rates obtained by prostaglandin based protocols, Select-Synch and different modifications ofOvsynch protocol were comparable to the results of Ovsynch. It was also indicated that in dairy herds with suboptimal detection of estrus a modification of Ovsynch protocol using presynchronization (Presynch) and TAI at the time of second GnRH injection could be an effective alternative for the hormonal manipulation of estrous cycle.

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HORMONALNE METODE ZA MANIPULACIJU ESTRUSNIM CIKLUSOM KOD MLEČNIH KRAVA

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Izvod

Efikasnost reprodukcije kod mlečnih krava ima tendenciju pogoršanja u svim delovima sveta pa i u Republici Srbiji. Epidemiološka ispitivanja ukazuju da se razlozi za smanjenje reproduktivne efikasnosti pre moraju tražiti u parametrima vezanim za pojavu različitih oboljenja nego u porastu proizvodnje mleka. Međutim, trenutno rešenje za problem smanjene plodnosti mlečnih krava najčešće se traži u primeni različitih hormonalnih metoda za manipulaciju estrusnim ciklusom kod mlečnih krava. Ultrazvučni pregled reproduktivnog trakta kod mlečnih krava je omogućio veterinarima da direktno prate promene na janicima i uterusu tokom estrusnog ciklusa, otvarajući prostor za pravovremenu primenu hormona radi inhibicije/stimulacije privremenih endokrinih struktura na jajnicima. U našem pokušaju da sumiramo najznačajnije hormonalne metode za manipulaciju estrusnog ciklusa kod mlečnih krava analizirali smo protokole bazirane isključivo na aplikaciji prostaglandina, metode zasnovane na kombinovanju prostaglandina i progestagena, režime manipulacije bazirane na primeni kombinacije prostaglandina i gonadotropnog oslobađajućeg hormona (GnRH), kao i primenu prostaglandina, GnRH i estradiola. U stadima mlečnih krava gde je optimalna detekcija estrusa metoda izbora za hormonalnu manipulaciju estrusnog ciklusa je tzv. Select Synch protokol. Ukoliko je na farmi ustanovljen prisutan problem detekcija estrusa može se primeniti Ovsynch protokol ili kombinacija Presynch i Ovsynch protokola za hormonalnu manipulaciju estrusnog ciklusa. Fiksno vreme veštačkog osemenjavanja koje se sprovodi u toku Ovsynch protokola može se pomeriti tako da se vrši u vreme druge aplikacije GnRH, čime se smanjuje broj fizičkih intervencija i skraćuje trajanje protokola.

Ključne reči: mlečne krave, estrusni ciklus, hormonalna manipulacija.

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