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Original article

Effect of ovulation rate and timing of ovulation after different hormone treatments on pregnancy rate in dairy cows

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Abstract

Primiparous and multiparous lactating crossbred dairy cows with a mature corpus luteum and a follicle with ≥10 mm in diameter were treated with cloprostenol. Those cows that showed oestrus within 5 days after treatment were inseminated (Group P). The other cows (Group PG) were treated with GnRH 2 days after cloprostenol treatment and timed artificial insemination (AI) was performed on the consecutive day, or were inseminated (Group G) after detected oestrus and treated with GnRH immediately after AI. The control cows (Group C) after detected oestrus were only inseminated. All of the AIs using frozen semen were done between 6 and 7 a.m. while the ultrasonographic examinations after AI were performed between 4 to 6 p.m. The ovaries of each cow were scanned by means of transrectal ultrasonography from the day of AI until ovulation. Daily blood samples were collected for progesterone measurements. The ovulation and pregnancy rates among the groups changed between 84.6% and 95.5%, as well as 44.4% and 60%, respectively, however the differences were not statistically significant.

All the cows were evaluated according to date of ovulation after AI and the pregnancy rate was 55.4% (Group 1: ovulation occurred between AI and 9-11 h after AI), 54.5% (Group 2: ovulation occurred between 9-11 h and 33-35 h after AI) and 35.5% (Group 3: ovulation occurred between 33-35 h and 57-59 h after AI), respectively. There was a trend (P=0.087) for 2.2 greater odds of staying open among cows inseminated between 33 to 35 h and 57 to 59 h before ovulation compared to cows inseminated within 9 to 11 h before ovulation. If ovulation occurred before AI, the pregnancy rate was only 22.2%, therefore determination of optimal time for AI is of great importance.

Key words: dairy cow, prostaglandin, GnRH, ultrasonography, progesterone

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Introduction

The initial time of oestrus/ovulation mainly depends on the follicular status when luteolysis has been induced by $PGF_{2\alpha}$ treatment (Odde 1990, Lucy et al. 1992, Roche and Mihm 1996). This variation could be attributed to the status of the actual follicular wave at the moment of treatment. It is well established by now that in most bovine oestrus cycles, two or three waves of follicular development occur. However, a small proportion of the cycles exhibit just one or alternatively four waves (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 would ovulate, resulting in a relatively short interval from treatment to ovulation, i.e. 2 to 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 to 5 days (Odde 1990, Lucy et al. 1992, Roche and Mihm 1996). Kastelic and Ginther (1991) found similar length for the interval from the day of treatment to the day of post treatment ovulation, depending on the follicular wave (1st wave: 4.2±0.1 days; 2nd wave: 6.3±0.3 days) at treatment. This great variation can also be confirmed by higher pregnancy rates, as have been achieved, when artificial insemination (AI) was performed after detected oestrus rather than that observed after timed AI (Kastelic and Ginther 1991).

Various attempts have been made to overcome this variability in response to prostaglandin treatments. With no benefits to the pregnancy rate but a better degree of synchronization if compared to untreated controls, this could be achieved by using a combined treatment of either progesterone, hCG or GnRH and prostaglandin. Pursley et al. (1995) advised a method of timed AI after GnRH and $PGF_{2\alpha}$ treatment. Ovulation was synchronized within an 8-h period, 24 to 32 h after the second treatment of GnRH, given after 2 days from $PGF_{2\alpha}$ treatment. The explanation of synchronization is that ovulation was possibly synchronized because preovulatory follicles were at a similar stage of growth and were responsive to LH at the time of the second GnRH treatment. Pregnancy rates after this synchronized ovulation were like those found in cows with a spontaneously detected oestrus (Pursley et al. 1997a).

On days 1 to 7 of the oestrous cycle, the size of corpus luteum (CL) increases (Kastelic et al. 1990b, Kastelic and Ginther 1991, Assey et al. 1993) and between days 8 to 14 CL reaches its maximal size (Tanabe and Hahn 1984, Kastelic et al. 1990b), while from day 14 to ovulation, its size gradually decreases (Kastelic et al. 1990b). The luteal tissue area and plasma progesterone concentration are highly correlated in heifers and cows (Sprecher et al. 1989, Kastelic et al. 1990a). A positive correlation was also observed between the size of CL as measured by ultrasonography and plasma P4 concentrations during cloprostenol induced luteal regression (Assey et al. 1993).

The aim of the present study was to evaluate the effect of different hormone treatments on the area of the follicle and CL, P4 concentration, timing of ovulation, and pregnancy rate in dairy cows.

Materials and Methods

Animals and treatments

This study was conducted at a North-east Hungarian dairy farm using a free stall housing system, with an average annual milk yield of 8200 kilograms per cow. Holstein-Friesian dairy cows, irrespective of their age, had a physiological sized uterus (the uterus was within the pelvic cavity) as described by Szenci et al. (1995), and optimal body condition score (3.0 to 3.5; using a scale from 0 to 5, according to Wildman et al. (1982). The farm uses pedometers (Afifarm Type: 4.1 04-016, AFIMILK Ltd. Israel) for oestrus detection. Artificial insemination (AI) of the cow in oestrus was carried out according to the a. m. - p.m. rule. Frozen semen used for AI was chosen by the herd managers as part of the routine management of the herd. According to the Hungarian standard one dosage of a frozen semen (0.5 ml) must contain more than 8 million viable sperm with progressive motility. The average conception rate for the first AI was 37.8 % during the period examined. All inseminated cows were examined for pregnancy by rectal palpation between days 45 and 75 after AI.

Primiparous and multiparous lactating crossbred dairy cows (after day 40 postpartum) with a mature CL (diameter of ≥ 17 mm determined by transrectal ultrasonography) without a cavity (Colazo et al. 2002, Répási et al. 2003) and having a follicle with a diameter of ≥ 10 mm were treated with 500 µg cloprostenol (n=80) (Estrumate, MSD Animal Health, Boxmeer, The Netherlands). Those cows that showed oestrus within 5 days after treatment were inseminated (Group P: n=39). The other cows in Group PG (n=22) after cloprostenol treatment were treated with 100 µg GnRH (Fertagyl, MSD Animal Health, Boxmeer, The Netherlands) 2 days later as suggested by Stevenson et al. (2003) and timed AI (TAI) was performed on the consecutive day irrespective of oestrus signs. Another 27 cows after detected oestrus (Group G) were inseminated and treated with 100 µg GnRH (Fertagyl) immediately after AI. Cows (n=73) after detected oestrus

Table 1. Effect of different hormone treatments on ovulation and pregnancy rate in dairy cows.

Treatment	AI (n)	Ovulation			Pregnant after AI n (%)	Pregnant after ovulation n (%)	P (relative odds ratio)
		before AI (n)	after AI (n)	no (n)			
Group P	39	0	33	6	19(48.7)	19(57.6)	0.289
Group PG	22	3	18	1	8(36.4)	8(38.1)	0.999
Group G	27	0	25	2	15(55.6)	15(60.0)	0.242
Group C	73	6*	62	5	30(41.1)	30(44.1)	Reference
Totals	161	9	138	14	72(44.7)	72(49.0)	

*Two cows became pregnant
Group P: treated with cloprostenol and inseminated within 5 days if oestrus was detected
Group PG: treated with cloprostenol (Day 0) and GnRH on Day 2, and timed AI was performed on Day 3
Group G: detection of oestrus + AI + GnRH
Group C (control): detection of oestrus + AI

were inseminated and served as a control group (Group C). All inseminations were done between 6 and 7 a.m. (Table 1) and double insemination was not performed.

The care of the animals and the experimental design of this study were approved by the Local Animal Ethics Committee in Budapest, Hungary.

Ultrasonographic examinations

Each ovary in the cows before cloprostenol treatment or before AI after detected oestrus (Day 0) was scanned between 6 to 7 a.m. using a real-time B-mode ultrasonographic scanner, operating with a 7.5 MHz linear-array transducer (Type 450, Pie Medical, Maastricht, The Netherlands) several times in lateromedial and dorsoventral planes to determine the position and the diameter (height, width) of the follicle and the CL, as described previously (Répási et al. 2003) and repeated after AI once daily between 4 to 6 p.m. in the afternoon until ovulation or latest 3 days after AI. When ovulation occurred, examinations were stopped and the area of the developing CL was not determined.

Blood sampling and progesterone determination

Heparinized blood samples were daily withdrawn from the jugular vein, starting immediately before AI and subsequently after each ultrasonographic examinations. Blood samples were collected until ovulation, or latest 57 to 59 h after AI in cows that did not ovulate. In Groups 1 to 3, blood samples were collected from every cow while in Group 4 only from 27 cows. Supernatant plasma was harvested after centrifugation (15 min at 1500 g), and stored at -20°C until assayed with a double-antibody solid phase RIA method in duplicate as described by Ranilla et al. (1994). 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 CL was considered to be non-functional, as suggested previously (Semambo et al. 1992).

Statistical analysis

All analyses were carried out by R 2.7.2. Statistical Software (R Development Core Team, 2007). The significant level was set at $p< 0.05$ and an effect was considered a trend when p was between 0.05 and 0.10.

Cows (n=138) were also grouped according to the day of ovulation after AI as follows: Group 1 cows showed ovulation within 9 to 11 h after AI, Group 2 cows showed ovulation within 33 to 35 h after AI, and Group 3 cows showed ovulation within 57 to 59 h after AI.

Differences in pregnancy rate among treatments (Groups P, PG, G and C, or Groups 1-3) were calculated using relative odds ratio (Fisher-test, R package epitools).

Differences in the area of the CL/follicle, and P4 concentration between treatments (Groups P, PG, G and C) and groups (Groups 1-3) on the day of AI and ovulation were analysed using general linear models. For multiple comparisons, the Tukey-test was used.

Results

The ovulation rates in the four treatment groups were as follows: 84.6%, 95.5%, 92.6% and 93.2%, respectively (Table 1). Although the lowest ovulation rate was detected in Group P when only cloprostenol treatment was used to induce oestrus, however, the differences among the groups were not statistically significant. The highest pregnancy rate (55.6%) was achieved when AI was performed together with a GnRH treatment (Group G) while the lowest (36.4%) in Group PG when fixed timed AI was performed after cloprostenol and GnRH treatment. At the same time none of the groups differed significantly from the control group

Table 2. Mean±SD values of the area of the largest follicle and the CL (if present) and the progesterone level on the day of AI and ovulation.

		N	Area of follicle (mm^2)	Area of CL* (mm^2)	Progesterone** (ng/mL)
ON THE DAY OF AI					
Group P	**all cows**	**33**	**212.2 ±68.1**	**127.2±45.1**	**0.25±0.25**
	open cows	14	229.6±66.9	138.1±61.4	0.25±0.17
	pregnant cows	19	199.4±67.9	122.3±38.2	0.25±0.30
Group PG	**all cows**	**18**	**183.4±99.8**	**112.0±58.2**	**0.26±0.10**
	open cows	10	165.6±71.7	102.1±41.5	0.28±0.11
	pregnant cows	8	205.8±128.7	123.9±77.4	0.24±0.09
Group G	**all cows**	**25**	**210.1±70.4**	**139.4±95.0**	**0.26±0.79**
	open cows	10	231.7±90.5	107.0 -	0.13±0.24
	pregnant cows	15	195.7±51.7	145.9±104.7	0.35±1.00
Group C	**all cows**	**62**	**207.7±62.2**	**128.6±66.1**	**0.37±1.19****
	open cows	34	210.5±62.0	146.6±73.6	0.51±1.42
	pregnant cows	28	204.3±63.3	92.7±25.0	0.06±0.03
ON THE DAY OF OVULATION					
Group P	**all cows**	**33**	**219.5±69.1** ^{a}	**122.5±33.1**	**0.18±0.19**
	open cows	14	232.2±61.0	179.1 -	0.16±0.15
	pregnant cows	19	210.2±74.8	111.2±20.2	0.19±0.22
Group PG	**all cows**	**18**	**142.7±70.2** ^{b}	**99.7±22.7**	**0.19±0.12**
	open cows	10	132.8±72.5	104.8±14.4	0.18±0.13
	pregnant cows	8	155.2±70.0	94.5±31.6	0.19±0.11
Group G	**all cows**	**25**	**203.1±86.0** ^{c}	**128.3±112.1**	**0.27±0.80**
	open cows	10	226.6±118.4	-	0.14±0.24
	pregnant cows	15	187.5±54.7	128.3±112.1	0.36±1.04
Group C	**all cows**	**62**	**213.5 ±73.8** ^{d}	**126.1±70.2**	**0.11±0.21**
	open cows	34	212.7±73.2	148.1±82.2	0.14±0.26
	pregnant cows	28	214.5±75.7	89.5±23.1	0.06±0.04

* n=51; ** n=27
^{ab} p=0.003, ^{bc} p=0.048, ^{bd} p=0.003

regarding the pregnancy rate. Somewhat higher pregnancy rate (38.1% to 60%) was achieved when it was calculated due to the occurrence of ovulation, however, these differences could also not reach a significant level (Table 1).

There were no significant differences in the area of the follicle among treatments on the day of AI, but on the day of ovulation, the area of the follicle in the open and pregnant cows (Group PG) was significantly lower compared to that in the other three groups (Group PG vs. P: p=0.003; Group PG vs. G: p=0.048; Group PG vs. C: p=0.003) (Table 2). However, there were no differences when open and pregnant cows were compared separately. There were also no significant differences in the area of the CL and P4 concentration among treatments on the day of AI or ovulation either.

It is important to mention that there were 9 cows ovulated before AI (Table 1) and only 2 of them became pregnant (22.2%).

Cows (n=138) ovulated after AI were evaluated according to the time of ovulation: showing ovulation within 9-11 h (Group 1: 53.6%), between 9-11 h and 33-35 h (Group 2: 23.9%) or between 33-35 h and 57-59 h after AI (Group 3: 22.5%), respectively (Table 3). The pregnancy rate in the three groups were 55.4%, 54.5% and 35.5%, respectively. There was a trend (p=0.087) for 2.2 greater odds of staying open among cows inseminated 57-59 h before ovulation (Group 3) compared to cows inseminated within 9-11 h of ovulation (Group 1). Pregnancy rates between Groups 1 and 2, or 2 and 3 did not differ significantly (p=0.999 in both cases). On the day of AI and ovulation,

Table 3. Evaluation of the pregnancy rate according to ovulation after AI in different treatment groups.

Groups	Group 1 (n)	Group 2 (n)	Group 3 (n)	Total
Group P (%)	16 (48.5%)	11 (33.3%)	6 (18.2%)	33 (100%)
Pregnant (%)	10 (62.5%)	6 (54.5%)	3 (50.0%)	19 (57.6%)
Group PG (%)	9 (50.0%)	3 (16.7%)	6 (33.3%)	18 (100%)
Pregnant (%)	5 (55.6%)	1 (33.3%)	2 (33.3%)	8 (44.4%)
Group G (%)	18 (72.0%)	3 (12.0%)	4 (16.0%)	25 (100%)
Pregnant (%)	12 (66.7%)	2 (66.7%)	1 (25.0%)	15 (60.0%)
Group C (%)	31 (53.6%)	16 (23.9%)	15 (22.5%)	62 (100%)
Pregnant (%)	14 (45.2%)	9 (56.3%)	5 (33.3%)	28 (45.2%)
Total (%)	74 (53.6%)	33 (23.9%)	31 (22.5%)	138 (100%)
Pregnant (%)	41 (55.4%)	18 (54.5%)	11 (35.5%)	70 (50.7%)

Group 1: ovulation occurred between AI and 9-11 h after AI
Group 2: ovulation occurred between 9-11 h and 33-35 h after AI
Group 3: ovulation occurred between 33-35 h and 57-59 h after AI

there were no significant differences among Groups 1, 2 and 3, or between the pregnant and open cows regarding the area of the follicle, the area of the CL or the plasma P4 concentration (Table 4). The pregnancy rate (49.4% vs. 52.9%) was also not influenced significantly by the presence or absence of the CL at AI.

It is important to mention that 4 of 73 cows in Group C (5.6%) at AI had higher than 1 ng/ml P4 concentration and all of them belonged to the group of cows being inseminated 57-59 h before ovulation (Group 3). Only one of them became pregnant, however, its P4 concentration was 1.04 ng/ml at AI compared with the other cows (2.3 ng/ml, 3.9 ng/ml and 5.8 ng/ml, respectively) which decreased to 0.54 ng/ml at 9-11 h after AI and 0.01 ng/ml at 33 to 35 h after AI.

Discussion

Although the lowest ovulation rate was detected in Group P when only cloprostenol treatment was used to induce oestrus and perform AI after detected oestrus, the pregnancy rate was somewhat higher than that in the control group (48.7% vs. 41.1%) and than those reported previously (42.7% /Gábor et al. 2004/, 38.9% / Pursley et al. 1997b/, 30.8% /Tóth et al. 2006), which confirms the usefulness of this protocol in dairy farms. This is also in agreement with the data obtained by Mialot et al. (1999) who suggested to use only prostaglandin treatment after calving and perform AI after detected oestrus in case of suboestrus if the oestrus detection on the farm is good.

Although the ovulation rate in Group PG was the highest (95.5%) among our treatment groups (84.6-93.2%), in spite of it the pregnancy rate became the lowest (36.4%) comparing with the other treatment groups (41.1-55.6%). Group PG also included the highest prevalence of cows that ovulated before AI (14.3%), however, none of these cows became pregnant (Table 1). Somewhat lower pregnancy rate (27.3%) was reported by Meyer et al. (2007), however, in their cases the average duration between GnRH treatment and TAI was 3 h. Although the sample size (n=22) was relatively small in our group compared to the other groups, however, it can be stated that randomly selected cows for cloprostenol and GnRH treatment cannot synchronize the time of ovulation as found previously (Stevenson et al. 2003), which may explain the lower pregnancy rate. Stevenson et al. (2003) also received conception rates not different from those in controls when administered GnRH 48 h after $PGF_{2\alpha}$ and used timed AI at 16-20 h later.

The highest pregnancy rate (55.6% vs. 41.1% in the control group) was detected in Group G when after detected oestrus and AI a GnRH injection was

Table 4. Mean±SD values of the area of the largest follicle and the CL (if present), and the progesterone concentration on the day of AI and ovulation (O).

		N	Area of follicle (mm^2)	Area of CL* (mm^2)	Progesterone** (ng/mL)
ON THE DAY OF AI					
Group 1	**all cows**	**74**	**200.6±58.8**	**104.7±52.7**	**0.21±0.51**
	open cows	33	210.8±59.7	98.2±21.3	0.15±0.18
	pregnant cows	41	192.3±57.5	107.2±61.3	0.27±0.68
Group 2	**all cows**	**33**	**214.1±74.3**	**143.5±59.3**	**0.20±0.22**
	open cows	15	216.3±82.7	141.9±53.7	0.24±0.17
	pregnant cows	18	212.2±68.9	145.2±68.2	0.17±0.25
Group 3	**all cows**	**31**	**210.6±91.1**	**129.2±69.5**	**0.57±1.27**
	open cows	20	207.1±81.0	139.9±84.4	0.70±1.52
	pregnant cows	11	216.8±111.4	110.1±26.7	0.29±0.34
ON THE DAY OF OVULATION					
Group 1	**all cows**	**74**	200.6±58.8	104.7±52.7	0.21±0.51
	open cows	33	210.8±59.7	98.2±21.3	0.15±0.18
	pregnant cows	41	192.3±57.5	107.2±61.3	0.27±0.68
Group 2	**all cows**	**33**	223.5±64.4	135.7±40.5	0.13±0.15
	open cows	15	225.4±65.6	179.1-	0.16±0.13
	pregnant cows	18	221.9±65.3	114.0±21.2	0.11±0.17
Group 3	**all cows**	**31**	190.7±118.7	188.1±83.5	0.14±0.24
	open cows	20	186.9±121.4	188.1±83.5	0.16±0.28
	pregnant cows	11	197.7±119.2	-	0.10±0.15

* n=51; ** n=103

given. According to a meta-analysis (Morgan and Lean 1993), treatment of dairy cows with GnRH immediately after AI have a positive effect on pregnancy rate, however, with the exception of repeated inseminations, while other studies were not able to confirm the benefit of such treatment after the first insemination (Stevenson et al. 1984).

At the same time, it is important to mention that the differences among the four groups regarding the ovulation and pregnancy rate were not statistically significant.

Walker et al. (1996) reported that the mean ovulation time relative to the first detected mount was 27.6±5.4 h and there was no difference between spontaneous and with $PGF_{2\alpha}$ induced estruses. In contrast, Van Eerdenburg et al. (2002) found that the highest ovulation rate was observed in a field study between 0 and 24 h after AI (56.2%) which is in agreement with the present findings (53.6%). Pursley et al. (1995) devised an Ovsynch protocol for timed AI which consisted of a GnRH treatment at a random stage of the oestrus cycle (Day 0), a $PGF_{2\alpha}$ treatment on Day 7 to induce luteolysis, and a second GnRH treatment on Day 9. In 20 cows, ovulation was synchronized within an 8-h interval, 24-32 h after the second injection of GnRH, however, some heifers (6 of 24) failed to ovulate (Pursley et al. 1995). Kaim et al. (2003) reported that 76% of the non-treated cows showed ovulation within 30 h, 12% between 30-50 h, and 12 % more than 50 h after the onset of oestrus, respectively. In our case, only 66.5% of the cows showed ovulations within 33-35 h after AI, while 19.3% ovulated between 33-35 h and 57-59 h after AI (Table 3). The prevalence rates of cows without ovulations were 10.2% in the treated groups and 6.9% in the control group, respectively, while ovulation occurred in 9 (5.5%) cows before AI.

In agreement with our previous findings after prostaglandin treatment (Répási et al. 2014), the highest pregnancy rate (55.6% to 66.7%) was achieved in the treated groups if ovulation occurred within 9-11 h after AI, while in Group C it was 45.2%. In each group the lowest pregnancy rate was found when ovulation occurred within 57-59 h after AI (Table 3). Cows inseminated within 57-59 h after AI (Group 3) stayed open with more than 2 times greater odds compared to cows inseminated on the day of ovulation (Group 1). According to Van Eerdenburg et al. (2002), pregnancy rate was 52% if the cow ovulated within 24 h after AI, while <20% became pregnant if ovulated after 48 h. Fricke et al. (2003) indicates that there is a 24-h window, in which AI can be conducted in relation to ovulation to increase pregnancy rate, however, to determine the optimal time for AI without synchronization of ovulation in the field needs further studies. In agreement with previous findings (Van Eerdenburg et al. 2002) if AI was performed before ovulation only 22.2% of cows became pregnant.

Regarding the area of the follicle, the area of the CL and the plasma P4 concentration there were no significant differences among the treatment groups on the day of AI (Répási et al. 2003), and with the exception of the area of the follicle on the day of ovulation, as well. Although significant differences (Group PG vs. Groups P and G) were detected only when pregnant and non-pregnant cows were compared together, while on the day of ovulation non-pregnant and pregnant cows had smaller area of the follicle compared to the other three groups. In agreement with previous findings (Colazo et al. 2015) the area of the ovulatory follicle did not affect the pregnancy rate.

On the day of AI as well as on the day of ovulation, there were also no significant differences among Groups 1 to 3 between the pregnant and open cows regarding the area of the follicle and the CL or the plasma P4 concentration (Répási et al. 2003).

Conclusion

Based on these results, if a CL can be detected during reproductive examination on a farm in which heat detection rate is poor, a shortened Ovsynch protocol can be recommended (Stevenson et al. 2003). By this way, one GnRH treatment and 7 days may be spared. The benefit of a shortened Ovsynch protocol in the field needs further investigations. Furthermore, it was observed that if cows ovulated too late comparing to the time of AI, conception rate was significantly lower, highlighting the importance of the determination of the optimal time for AI. In case of US follow-up of ovaries, if ovulation does not occur within two days after AI, a second AI could be performed, however, further studies have to be conducted in order to evaluate the benefits of a second AI.

References

Assey RJ, Purwantara B, Greve T, Hyttel P, Schmidt MH (**1993**) Corpus luteum size and plasma progesterone levels in cattle after cloprostenol-induced luteolysis. Theriogenology 39: 1321-1330.

Colazo MG, Behrouzi A, Ambrose DJ, Mapletoft RJ (**2015**) Diameter of ovulatory follicle at timed artificial insemination as a predictor of pregnancy status in lactating dairy cows subjected to GnRH-based protocols. Theriogenology 84: 377-383.

Colazo MG, Martínez MF, Kastelic JP, Mapletoft RJ (**2002**) Effects of dose and route of administration of cloprostenol on luteolysis. estrus and ovulation in beef heifers. Anim Reprod Sci 72: 47-62.

Fricke PM, Caraviello DZ, Weigel KA, Welle ML (**2003**) Fertility of dairy cows after resynchronization of ovulation at three intervals following first timed insemination. J Dairy Sci 86: 3941-3950.

Gábor G, Tóth F, Szász F, Petró T, Györkös I (**2004**) Ways of decrease the period between calvings in dairy cows. 2. Oestrus induction and ovulation synchronisation methods. Magyar Állatorv Lapja 126: 658-663.

Ginther OJ, Knopf L, Kastelic JP (**1989a**) Temporal associations among ovarian events in cattle during oestrus cycles with two and three follicular waves. J Reprod Fertil 87: 223-230.

Ginther OJ, Kastelic JP, Knopf L (**1989b**) Compositions and Characteristics of follicular waves during the bovine estrous cycle. Anim Reprod Sci 20: 187-200.

Kaim M, Bloch A, Wolfenson D, Braw-Tal R, Rosenberg M, Voet H, Folman Y (**2003**) Effects of GnRH administered to cows at the onset of estrus on timing of ovulation, endocrine responses, and conception. J Dairy Sci 8 6: 2012-2021.

Kastelic JP, Ginther OJ (**1991**) Factors affecting the origin of the ovulatory follicle in heifers with induced luteolysis. Anim Reprod Sci 26: 13-24.

Kastelic JP, Bergfelt DR, Ginther OJ (**1990a**) Relationship between ultrasonographic assessment of the corpus luteum and plasma progesterone concentration in heifers. Theriogenology 33: 1269-1278.

Kastelic JP, Pierson RA, Ginther OJ (**1990b**) Ultrasonic morphology of corpora lutea and central luteal cavities during the estrous cycle and early pregnancy in heifers. Theriogenology 34: 487-498.

Knopf L, Kastelic JP, Schallenberger E, Ginther OJ (**1989**) Ovarian follicular dynamics in heifers: test of two-wave hypothesis by ultrasonically monitoring individual follicles. Domest Anim Endocrinol 6: 111-119.

Lucy MC, Savio JD, De Badinga L, La Sota RL, Thatcher WW (**1992**) Factors that affect ovarian follicular dynamics in cattle. J Anim Sci 70: 3615-3626.

Meyer JP, Radcliff RP, Rhoads ML, Bader JF, Murphy CN, Lucy MC (**2007**) Timed artificial insemination of two consecutive services in dairy cows using prostaglandin F2alpha and gonadotropin-releasing hormone. J Dairy Sci 90: 691-698.

Mialot JP, Laumonnier G, Ponsart C, Fauxpoint H, Barassin E, Ponter AA, Deletang F (**1999**) Postpartum subestrus in dairy cows: comparison of treatment with prostaglandin F2alpha or GnRH + prostaglandin F2alpha + GnRH. Theriogenology 52: 901-911.

Morgan WF, Lean IJ (**1993**) Gonadotrophin-releasing hormone treatment in cattle: a meta-analysis of the effects on conception at the time of insemination. Aust Vet J 70: 205-209.

Odde KG (**1990**) A review of synchronization of estrus in postpartum cattle. J Anim Sci 68: 817-830.

Pursley JR, Kosorok MR, Wiltbank MC (**1997a**) Reproductive management of lactating dairy cows using synchronization of ovulation. J Dairy Sci 80: 301-306.

Pursley JR, Wiltbank MC, Stevenson JS, Ottobre JS, Garverick HA, Anderson LL (**1997b**) Pregnancy rates per artificial insemination for cows and heifers inseminated at a synchronized ovulation or synchronized estrus. J Dairy Sci 80: 295-300.

Pursley JR, Mee MO, Wiltbank MC (**1995**) Synchronization of ovulation in dairy cows using PGF2alpha and GnRH. Theriogenology 44: 915-923.

Ranilla MJ, Sulon J, Carro MD, Mantecón AR, Beckers JF (**1994**) Plasmatic profiles of pregnancy-associated glycoprotein and progesterone levels during gestation in Churra and Merino sheep. Theriogenology 42: 537-545.

Répási A, Beckers JF, Sulon J, Perényi Z, Reiczigel J, Szenci O (**2003**) Effect of different doses of prostaglandin on the area of corpus luteum, the largest follicle and progesterone concentration in the dairy cow. Reprod Domest Anim 38: 423-428.

Répási A, Szelényi Z, Reiczigel J, Bajcsy AC, Horváth A, Szenci O (**2014**) Control of ovulation after prostaglandin treatment by means of ultrasonography and effect of the time of ovulation on conception rate in dairy cows. Acta Vet Hung 62: 74-83.

Roche JF, Mihm M (**1996**) Physiology and practice of induction and control of estrus in cattle. Proc XIX World Buiatrics Congress, Edinburgh, pp. 157-163.

Savio JD, Keenan L, Boland MP, Roche JF (**1988**) Pattern of growth of dominant follicles during the oestrous cycle of heifers. J Reprod Fertil 83: 663-671.

Semambo DK, Eckersall PD, Sasser RG, Ayliffe TR (**1992**) Pregnancy-specific protein B and progesterone in monitoring viability of the embryo in early pregnancy in the cow after experimental infection with Actinomyces pyogenes. Theriogenology 37: 741-748.

Sirois J, Fortune JE (**1988**) Ovarian follicular dynamics during the estrous cycle in heifers monitored by real-time ultrasonography. Biol Reprod 39: 308-317.

Sprecher DJ, Nebel RL, Whitman SS (**1989**) The predictive value, sensitivity and specificity of palpation per rectum and transrectal ultrasonography for the determination of bovine luteal status. Theriogenology 31: 1165-1172.

StatSoft I. STATISTICA (data analysis software system). Version 6.1. 1984-2004. StatSoft. Inc.: Tulsa. OK. USA. www.statsoft.com

Stevenson JS, Cartmill JA, Hensley BA, El-Zarkouny SZ (**2003**) Conception rates of dairy cows following early not-pregnant diagnosis by ultrasonography and subsequent treatments with shortened Ovsynch protocol. Theriogenology 60: 475-483.

Stevenson JS, Schmidt MK, Call EP (**1984**) Gonadotropin--releasing hormone and conception of Holsteins. J Dairy Sci 67: 140-145.

Szenci O, Gyulai G, Nagy P, Kovács L, Varga J, Taverne MA (**1995**) Effect of uterus position relative to the pelvic inlet on the accuracy of early bovine pregnancy diagnosis by means of ultrasonography. Vet Q 17: 37-39.

Tanabe TY, Hahn RC (**1984**) Synchronized estrus and subsequent conception in dairy heifers treated with prostagtlandin F2alpha. I. Influence of stage of cycle at treatment. J Anim Sci 58: 805-811.

Tóth F, Gábor G, Mézes M, Váradi É, Ózsvári L, Sasser RG, Abonyi-Tóth Z (**2006**) Improving the reproductive efficiency by zoo-technical methods at a dairy farm. Reprod Domest Anim 41: 184-188.

Van Eerdenburg FJ, Karthaus D, Taverne MA, Merics I, Szenci O (**2002**) The relationship between estrus behavioral score and time of ovulation in dairy cattle. J Dairy Sci 85: 1150-1156.

Walker WL, Nebel RL, McGilliard ML (**1996**) Time of ovulation relative to mounting activity in dairy cattle. J Dairy Sci 79: 1555-1561.

Wildman EE, Jones GM, Wagner PE, Boman RL, Troutt, HFJ, Lesch TN (**1982**) A dairy cow body condition scoring system and its relationship to selected production characteristics. J Dairy Sci 65: 495-501.