

RESULTS OF APPLYING GONADORELIN FERTAGYL ON THE PROGESTERONE CONCENTRATIONS IN THE BLOOD SERUM AND COW CONCEPTION

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Abstract: Considering there are different and inconsistent results of the wider use of gonadotropin-releasing hormone or its analogs in cattle breeding and due to scarce research studies in the field of individual breeding, we decided to explore the influence of gonadorelin Fertagyl on the results of the blood serum progesterone levels and conception of cows in veterinary field practice. Examination of the progesterone levels involved 14 (fourteen) cows treated with gonadorelin (GnRH), Fertagyl, manufactured by Intervet, according to the manufacturer's instructions dose of 2,5ml (250 micrograms), within five minutes after insemination. There are individual differences in progesteron concentrations according to days of sampling in treated cows, respectively individuality is manifested in all cows between days of blood sampling, except for period of luteolysis (18th day) in non-gravid cows. Our research results indicate that there is a tendency for progesterone concentration to increase in gravid cows treated with Fertagyl at the time of insemination. Progesteron concentrations in blood serum in gravid cows were higher for 0.21 ng/ml in first three days after insemination and at 6th day those concentrations were higher for 0.40 ng/ml compared to non-gravid cows.

Key words: Fertagyl, progesterone, blood serum, cow

Introduction

Considering there are different and inconsistent results of the wider use of gonadotropin-releasing hormone or its analogs in farm breeding and due to scarce research studies in the field of individual breeding, we decided to explore the

influence of gonadorelin Fertagyl on the results of the blood serum progesterone levels and conception of cows in veterinary field practice. The blood of treated cows was sampled to examine the level of progesterone concentration in the serum of cows.

Materials and Methods

Examination of the progesterone levels concentration involved 14 (fourteen) cows treated with gonadorelin (GnRH), Fertagyl, manufactured by Intervet, according to the instructions provided by the manufacturer in dose of 2,5 ml (250 micrograms) and 14 (fourteen) cows treated with physiological solution (placebo). After taking the anamnestic data from the owners, such as the time of onset of estrus, and after performing gynecological examination of cows, Fertagyl was applied five minutes after insemination. The research included cows brought in for insemination for the first time, mostly 50-80 days after calving, and those that were re-entering estrus for the second or third time. Blood sampling was performed 3, 6, 9, 12, 15 and 18 days after insemination, that is, after the treatment with gonadorelin. The blood for determining the level of progesterone was taken by puncturing the tail vein with vacuum syringe. Following the blood sampling, serum was taken out and stored at -20 degrees C until the time of testing. Radioimmunoassay method (RIA) as well as commercial packages were used to determine the level of progesterone concentration. Radioactivity of samples was at all times measured under the same conditions using Gama Scintillation counter. The results were calculated according to the instructions provided by the reagent's manufacturer. The diagnosis of gestation was performed 50 to 60 days after insemination.

Results and Discussion

The levels of progesterone relative to the number of the serum samples from 14 (fourteen) cows show significant individual discrepancies in the level of progesterone concentration in relation to the days of sampling. The progesterone concentrations ranged from the minimum value of 0.80 ng/ml to the maximum value of 1.64 ng/ml in the first three days. On day 6 of sampling, there are also significant variations, however, of some lesser degree. In the majority of cows, except in cow 6 and 10, more equal elevation of the concentration level begins after day 6 until day 15.

Table 1. Levels of progesterone concentration (ng/ml) in the blood serum of gravid cows treated with GnRH (Fertagyl)

Number of cows	Blood sampling days after insemination					
	3	6	9	12	15	18
1	0.94	2.83	7.00	7.18	6.80	7.00
2	1.64	3.90	6.20	6.50	5.70	6.10
3	1.50	3.54	5.00	9.42	8.80	9.00
4	1.24	5.52	7.88	7.60	6.90	7.50
5	1.30	4.78	6.00	7.00	7.20	6.50
6	1.39	2.10	2.97	3.68	3.75	5.58
8	1.62	3.20	6.70	7.41	6.56	6.91
9	1.40	1.51	3.93	4.38	6.15	9.19
10	0.81	0.85	2.42	2.52	2.63	4.37
14	1.30	3.10	4.90	7.00	6.80	5.90
TOTAL	13.14	31.33	53.00	62.69	61.29	70.05
Average	1.31	3.13	5.30	6.26	6.13	7.00

The serum progesterone concentrations in the blood serum of gravid cows were higher by 0.21 ng/ml in the first three days after insemination, and on day 6, the concentrations were higher by 0.40 ng/ml in relation to non-gravid cows. In addition, there are large individual discrepancies in progesterone concentration in all gravid cows during all of the time periods of blood sampling. Initial concentrations of progesterone in some cows were very low all the time until the recognition of gravidity, when they started to elevate (cow 6 and 10).

Our results of the progesterone testing study also suggest there is a tendency for concentrations of progesterone to increase in gravid cows treated with Fertagyl at the time of insemination. There is appearance of an increasing concentration of progesterone in the blood of the cows treated with Fertagyl, which manifests on day 3 and 6 of blood sampling, as the individual discrepancy in progesterone concentration is very large during all sampling times.

Table 2. Levels of progesterone concentration (ng/ml) in the blood serum of non-gravid cows treated with gonadorelin (Fertagyl)

Number of cows	Blood sampling days after insemination					
	3	6	9	12	15	18
7	1.20	2.93	5.89	7.23	6.73	3.43
11	1.10	2.80	4.70	5.60	6.20	4.40
12	0.80	2.20	4.80	5.34	4.71	3.70
14	1.30	3.10	4.90	7.00	6.80	5.90
TOTAL	4.40	11.03	20.29	25.17	24.40	17.43
Average	1.10	2.75	5.07	6.29	6.11	4.35

As in gravid cows, there is also a gradual increase in concentration of progesterone in relation to the sampling days, except on day 18, when there is a significant decline due to the onset of luteolytic activity of prostaglandin from uterus.

Table 3. Levels of progesterone concentration (ng/ml) in the blood serum of gravid cows treated with placebo

Number of cows	Blood sampling days after insemination					
	3	6	9	12	15	18
1	1.30	2.33	5.08	5.13	6.71	7.96
4	1.37	3.56	3.98	4.76	5.06	7.95
5	0.04	0.15	4.50	5.60	5.06	5.40
8	1.10	2.76	3.96	4.52	4.47	4.97
9	1.20	2.30	4.50	5.67	5.76	6.16
10	1.52	2.02	2.04	2.35	4.26	6.02
11	0.72	1.42	4.00	6.10	6.30	6.00
12	1.20	2.30	4.40	6.50	5.90	5.20
TOTAL	8.51	16.84	32.46	40.63	44.52	49.66
Average	1.06	2.10	4.05	5.07	5.56	6.20

It is evident that the median concentrations of progesterone relative to the time of blood sampling are similar during the equivalent sampling periods, with quite a proper trend, except in cow 10, whose levels of the progesterone

concentration were low all the time until day 15. The levels of progesterone concentrations were also rather high on day 18 of sampling.

Table 4. Levels of progesterone concentration (ng/ml) in the blood serum of non-gravid cows treated with placebo

Number of cows	Blood sampling days after insemination					
	3	6	9	12	15	18
2	0.45	1.13	3.47	5.65	7.65	3.87
3	0.01	0.18	0.20	0.27	0.74	0.01
6	1.48	2.65	6.29	7.49	6.98	3.50
7	0.84	1.35	1.99	2.10	2.16	2.98
13	1.00	2.90	3.80	5.40	6.00	3.90
14	0.90	3.40	5.40	6.30	6.10	3.50
TOTAL	4.68	11.61	21.15	27.21	29.63	17.76
Average	0.78	1.93	3.52	4.53	4.97	2.96

Although, the average picture of progesterone secretion indicates the gradual period of elevation, maintenance and the degree of decline in hormonal secretion, the profiles of progesterone concentration indicate that there is individual variation between days of blood sampling, in fact, except for the period of luteolysis (day 18) in non-gravid cows, when there is a more significant decline in progesterone concentration. Very low concentrations of progesterone were observed in three cows (2, 4, and 7) all the time until day 9 after insemination.

Sexual cyclicity of cows is stimulated through the *hypothalamus-pituitary-ovary-uterus* mechanism. Ovulation comes at the end of estrus, as well as formation of the “yellow body” – *Corpus luteum* (plural *Corpora lutea*). Luteal cells of „yellow body“ are glandular cells which produce steroids, respectively luteal cells excrete second female sexual hormone Progesterone. Formation of „yellow body“ is under influence LH and excretion of Progesterone is under influence LTH (*Katica et al., 2010*). Secretion of progesterone stops abruptly several days before the next estrus. The period of *corpus luteum* activity represents the luteal phase that lasts 16 to 17 days in cows, while the follicular phase lasts from 3 to 6 days. The picture of the blood progesterone level in cows during the sexual cycle is well defined. The progesterone levels are at the minimum values around the time of estrus < 0.5 ng/ml; on day 4 after estrus, the concentrations are gradually starting to elevate up to the maximum values, from 4 to 13 ng/ml between day 8 and day 15. After that, the levels of progesterone concentrations drop down to the initial basal values, some two days before estrus (*Pope et al., 1969; Schams et al., 1989*). According to *Schamberger et al., (1967)*, the average

concentration of progesterone on days 6, 11, 16 and 21 is 3; 4, 8; 9.6; and 1.5 ng/ml. *Gupta and Pope, (1968)* published about cyclical level of progesterone concentrations in non-gravid cows. The progesterone concentrations in luteal phase ranged from 7.5 to 10 ng/ml and from 1 to 2 ng/ml around day 4 to day 6 before the ovulation. According to *McCracken (1991)*, in two cows, during two consecutive sexual cycles, the average progesterone concentration in plasma on day 12 of the sexual cycle was 9.6 and 8.8 ng/ml.

Stabenfeldt et al., (1969) point out that the levels of progesterone vary from 0.4 ng/ml in the blood plasma at the time of estrus to approximately 7 ng/ml at the peak of the luteal phase of the sexual cycle (range from 6.1 to 10.2 ng/ml). The decline on day 18 continued until the next sexual cycle, and the first significant elevation in the next cycle occurred on day 4. According to *Garverick et al., (1971)*, the progesterone levels are at the minimum value around estrus, < 0.5 ng/ml, on day 4 after estrus; the concentrations are gradually elevating, reaching the level of 4 to 13 ng/ml between day 8 and day 15 of the cycle, and after that, they drop down to the basal values some two days before next estrus cycle.

The progesterone concentrations in plasma during the first 14 days after estrus are similar regardless of whether the animal conceived or not (*Pope et al., 1969*), and after that, the values in non-gravid cows drop down; however, in gravid animals, the values are either maintained or elevated, staying at the level of around 9 ng/ml between day 30 and day 80. Studying the successfulness of the GnRH application in cows re-entering estrus in the field conditions in relation to the time intervals of the onset of estrus (6 to 8, 9 to 12 hours and longer), the results of conception were significantly better in treated cows in relation to controlling cows, if insemination and application of gonadorelin were performed within 6 to 8 hours after the first signs of estrus (77.41%:56.06%), (*Mutevelić et al., 2003*). The levels may fluctuate during the gravidity.

Ferizbegović (1995) points out that there are very significant individual discrepancies in the progesterone concentration levels in the serum of cows from highland and mountainous area. The discrepancies manifested particularly in relation to the time of duration of the first sexual cycles after calving, post partum. The author also observed that there were significant individual discrepancies in the maximum concentrations of progesterone in relation to the length of sexual cycles. The maximum concentrations of progesterone in short sexual cycles range from 1.92 to 3.77 ng/ml; in normal sexual cycles, they range from 3.58 to 8.10 ng/ml and from 2.50 to 7.10 ng/ml in long cycles.

Endogenous appearance of luteinizing hormone (LH) during estrus is significant and vital for ovulation and luteinization of granulosa and thecal cells, whose production of progesterone is necessary for maintaining of gestation (*Henderson 1979*). Injection of GnRH at the time of artificial insemination 10 hours after the first sign of estrus was able to induce additional appearance of LH (*Lee et al. 1985*). LH secretion and dynamics of follicular development after

application of gonadorelin, had been monitored by Marcelo et al., 2003 in their own researches.

The cows that responded to GnRH with median LH had larger production of progesterone than other groups. This was recorded as early as first four days after insemination. Hence, the higher progesterone in cows treated with GnRH, which were gravid, was likely due to appearance of LH after the application of GnRH, causing more of granulosa cells to become luteal cells for the production of progesterone or due to the improvement in the production of progesterone by the existing cells. Furthermore, it was announced that there was a significant relationship between the progesterone concentration and gravidity in heifers. The importance of progesterone concentration for maintaining gravidity was elaborated (Lee et al., 1985), which points out that 14% of cows in an early period post partum did not produce enough progesterone after ovulation to maintain gravidity, if there was conception. The progesterone concentrations during sexual cycle before ovulation may also be the indicator of later fertility in cows (Fonesca et al., 1983).

Our results prove that the treatment during the insemination of cows re-entering estrus may improve fertility, which is in relation with results of Leslie and Kelton (1992), which during research of gonadorelin (GnRH) application in the time of artificial insemination in 93 dairy herd in Ontario, Canada, determined that there is improvement of conception in cows that three or more times need to be re-breed. It should not ignore the possibility that the percentage of conception is improved by repeatedly treating the same cow with Fertagyl during consecutive insemination. It was determined that repeat-breeder cows or heifers needed to be inseminated three or more times in order to successfully conceive and that they had normal interestrus interval and anatomic normal reproductive tract, (Zobel et al., 2011). The treatment with GnRH during estrus is likely to affect the time of ovulation, the fertility, the development of *corpus luteum*, the secretion of progesterone and the survival of embryo, (Jadav et al., 2010). In terms of conception it should not forget anti-luteolytic activity of bTP-1 (bovine trophoblast protein complex). The higher degree of conception in cows treated with GnRH is likely the result of an increased production of progesterone, which maintains gravidity. Treatment with GnRH during estrus probably influences, among other things, the development of *corpus luteum* and the secretion of progesterone.

Conclusions

- There is the tendency for progesterone concentrations to increase in the blood of gravid cows treated with Fertagyl, which manifests on day 3 and 6 of blood sampling, as the individual discrepancy in

concentrations of progesterone is very large during all sampling periods in both controlling and treated cows.

- A quantitative tendency of increasing concentrations of progesterone in the blood of gravid cows treated with Fertagyl is observed in gravid cows in relation to the sampling days, except on day 18, when there is a significant decline due to the onset of luteolytic activity of prostaglandin from uterus.
- The progesterone concentrations in plasma during the first 14 days after estrus are similar regardless of whether the animal conceived or not and after that, the values in non-gravid cows drop down; however, in gravid animals, the values are either maintained or elevated, staying at the level of around 9 ng/ml between day 30 and day 80.
- It seems that injection of GnRH at the time of artificial insemination (10 hours after the first sign of estrus) is able to induce additional secretion of LH.
- Cows that responded to GnRH with median LH had larger production of progesterone than other groups.
- The treatment with GnRH during estrus is likely to affect the time of ovulation, the fertility, the development of *corpus luteum*, the secretion of progesterone and the survival of embryo.
- The higher degree of conception in cows treated with GnRH is likely the result of an increased production of progesterone, which maintains gravidity.
- Treatment with gonadorelin during the insemination of cows re-entering estrus may improve fertility.

Rezultati aplikacije gonadorelina Fertagyl na koncentracije progesterona u krvnom serumu i koncepciju krava

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Rezime

Uzimajući u obzir postojanje različitih i protivrečnih rezultata u široj upotrebi gonadotropnog-oslobađajućeg hormona ili njegovih analoga u uzgoju goveda i usled oskudnih istraživanja na polju pojedinačnog uzgoja, odlučili smo istražiti uticaj gonadorelina Fertagyl na rezultate nivoa progesterona u krvnom serumu i koncepciju krava u veterinarskoj terenskoj praksi.

Pregled nivoa progesterona je uključivao 14 (četnaest) krava tretiranih sa gonadorelinom (GnRH) Fertagyl, od proizvođača Intervet i to prema uputstvu u dozi od 2,5 ml (250 mikrograma), unutar 5 minuta nakon osemenjavanja. Postoje individualne razlike u koncentracijama progesterona u odnosu na dane uzorkovanja u tretiranih krava, odnosno individualnost se manifestuje u svih krava između dana uzorkovanja krvi, osim za period luteolize (18. dan) kod negravidnih krava.

Naša istraživanja ukazuju da postoji težnja da koncentracije progesterona porastu kod gravidnih krava tretiranih sa Fertagyl u vremenu osjemenjavanja. Koncentracije progesterona u serumu gravidnih krava bile su veće za 0,21 ng/ml u prva tri dana nakon osjemenjavanja, a 6. dana te koncentracije su bile više za 0,40ng/ml u odnosu na negravidne krave.

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