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STUDY ON THE PERFORMANCE OF GONADOTROPIN-RELEASING HORMONE (GnRH) IN THE PUERPERAL PERIOD OF BEEF COWS

Ts. Maslev, Ts. Hristova*, S. Stoycheva

Institute of Mountain Stockbreeding and Agriculture, 281 Vasil Levski Str., Troyan, Bulgaria
*Corresponding author: cvetomira_16@abv.bg
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Abstract: The effect of the intravenous injection of 100 mcg (2 ml) gonadotropin-releasing hormone (Ovarelin, Ceva) from the 1st to the 20th day after calving of beef cows was monitored in the article, in regard to the occurrence of the first oestrus and the possibilities for its controlling. It was established that in the first minutes of intravenous application of Ovarelin the concentration of luteinizing hormone (LH) increased. By increasing the number of days increased the amount of secreted LH. Maximum effect of the injection of 100 mcg GnRH occurred between the 10th and 15th day. The physiological dose (100 mcg) GnRH injected intravenously on the 1st day after birth of cows led to increased content of LH in blood circulation.

Key words: puerperal period, beef cows, gonadotropin hormones

Introduction

It is known that the successful reproduction is a result of a number of endocrine, paracrine, follicular and gametogenesis factors. The pattern of folliculogenesis after birth is a series of physiological phenomena involving the growth of germinal and somatic cells, creation, differentiation, atresia and ovulation of the dominant follicle *(Roche and Diskin, 1995)*.

Timely insemination of cows in the postpartum period shortens the period between calvings and increases efficiency in their breeding. Pregnancy and the duration of the service period are the components defining this. Recovery of normal ovarian function after puerperium and getting each year one calf from a cow is determined by a number of other factors. They are the following: the course of the birthing process, nutrition, milk yield, sucking of the calf and the presence of male animals *(Kawashima et al., 2008).*

It is found that after the calving the dominant follicle is temporarily suppressed, which is normally obtained during part of the oestrous cycle and during pregnancy but not all factors are completely known to us *(Hirshfield, 1994).*

Therefore, knowledge of the hormonal dynamics in the puerperium is a precondition for obtaining better results in cattle breeding. The process of maturing of follicles and the first ovulation occurs within the first 45 days, but in the majority of animals that is not accompanied by clinical expression of oestrus *(Sheldon, 2004).* Adenohypophyseal hormones and the stimulating effect on them of hypothalamic gonadotropin-releasing hormone (GnRH) have essential importance for these processes. After synthesis of GnRH, it has found wide application in practice. The effects of various pharmacological doses (over 100 mcg) have been studied comparatively well. But the question of the influence of smaller doses and their physiological performance has not been elucidated yet.
In this regard the main objective of performed experiment was to investigate the possibility of shortening the postpartum period by the application of physiological doses GnRH (100 mcg).

Material and Methods

The study was conducted in the Experimental base in the Institute of Mountain stockbreeding and agriculture in Troyan, using 15 cows from Limousine breed, (multiparous) divided into 5 groups. Calving seasons at spring. Each of the groups was injected with 100 mcg GnRH (2 ml, Ovarelin – Ceva) intravenously, on the 1^{st}, 5^{th}, 10^{th}, 15^{th} and 20^{th} day after calving respectively. Blood samples were obtained by puncture of the opposite vena jugularis in the 1^{st}, 5^{th}, 10^{th}, 15^{th}, 20^{th}, 30^{th} and 120^{th} minutes after injection, respectively. It was taken 10 ml of blood in pre-heparinized test tubes. Blood plasma was exuded after centrifugation at 3000 rpm, per 10 minutes.

The values of LH hormone were determined by radio-immunological methods Beckman LS-800, by marking with I^{125}.

Results and Discussion

The changes in the serum concentration of LH in cows treated with 100 mcg GnRH at different days after birth are shown in Figure 1.

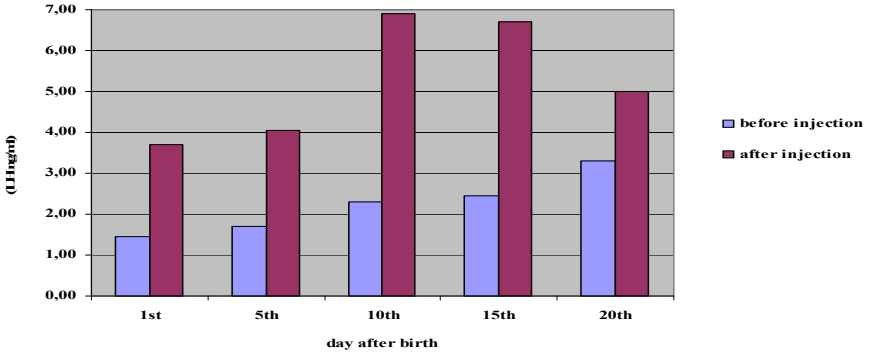

Figure 1. Concentration of LH (ng/ml) before and after *i/v* injection of 100 mcg GnRH (Ovarelin) (Summarized values)

Chart of the average values of LH in the particular groups of animals allows monitoring of the changes in the reactivity of the hypophysis during the first 20 days after calving birth. The measured amount of LH of the group treated with 100 mcg GnRH on the 1st day after calving was 1.20±0.35 ng/ml (Figure 2).

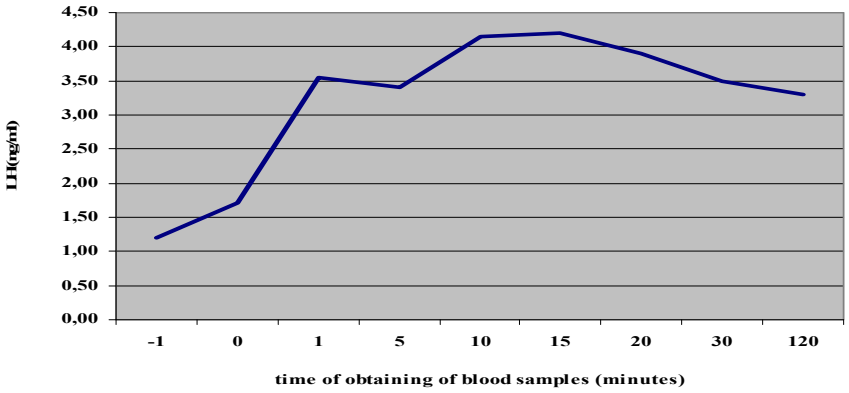

Figure 2. Amount of LH (ng/ml) in blood plasma serum of beef cows, in the 1st day after parturition birth

Already in the first minute after the intravenous injection of 2 ml Ovarelin (100 mcg GnRH), the values of the hormone started to rise – 3.55±1.20 ng/ml, nevertheless that the amounts in the individual animals varied greatly. Maximum measured values of LH 4.20±0.45ng/ml were established in the 15th minute. Then its amount decreased and in the 120th minute was 3.30±0.87 ng/ml.

As a whole, the data showed that the response of hypophysis regarding the quantitative secretion of LH was comparatively weak.

The LH level was 1.53±0.89 ng/ml in cows treated on the 5th day after calving (Figure 3).

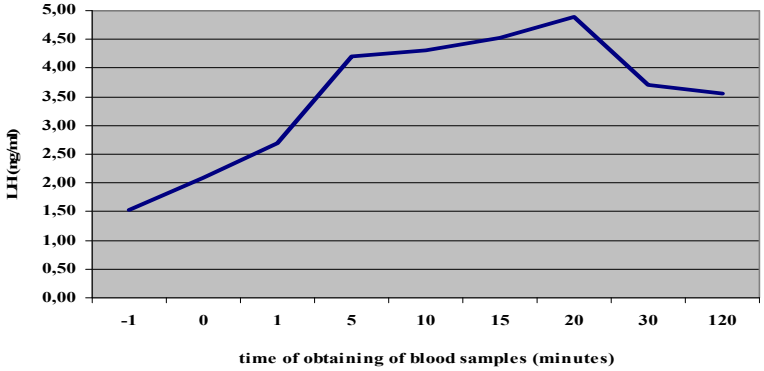

Figure 3. Amount of LH (ng/ml) in blood plasma serum of beef cows, in the 5th day after parturition birth

In comparison to the results obtained in cows treated on the 1st day after calving, in this group in all animals was established increasing of values, 2.69±1.17 ng/ml in 1st minute, 4.21±1.09 ng/ml in 5th minute, 8.14±0.80 ng/ml in 10th minute, 6.09±0.74 ng/ml in 15th minute and 3.22±0.41 ng/ml in 20th minute. Monitoring the values till the 120th minute showed that the amount of LH decreased (3.55±1.15 ng/ml). Higher were the basal values in cows in the group treated on the 10th day after calving (2.39±0.28 ng/ml). Maximum values of LH were measured in the 15th minute after the injection (9.50±1.01 ng/ml). On the 15th day after the treatment, in the basal values of 2.40±0.50 ng/ml, the total amount of LH was decreased – average 6.70±0.56 ng/ml, as the highest value was in the 15th minute 8.29±1.40 ng/ml (Figure 4).

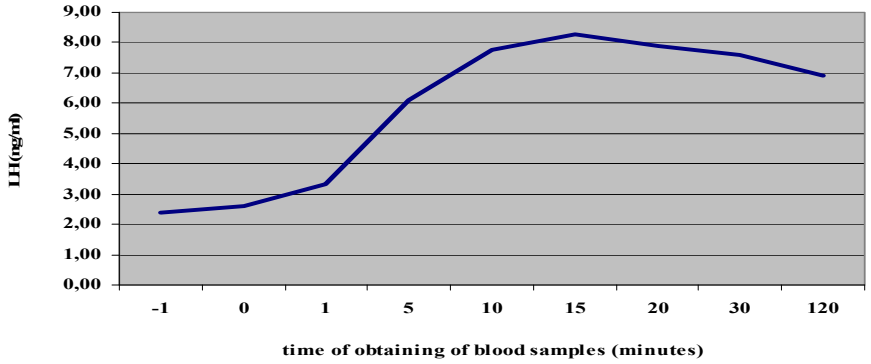

Figure 4. Amount of LH (ng/ml) in blood plasma serum of beef cows, in the 15th day after parturition birth

On the 20th day the amounts of LH were 3.30±0.32 ng/ml – basal and 5.01±0.33 ng/ml – average, respectively.

It was found that in all cows involved in the experiment, at higher values of LH before calving, were obtained higher values after treatment in different periods of the puerperium.

The obtained results showed that the pituitary gland reacted with increasing of LH production immediately after injection of GnRH, and the highest average values were obtained on the 10th day (6.89±0.57 ng/ml).

Number of authors found that after calving there were formed ovarian follicles which estrogen hormones had primary role in gonadotropin secretion *(Kaltenback et al., 1974; Kesler et al., 1977)*.

These estrogens influencing on the hypophysis increased its reactive sensitivity to exogenously applied GnRH. This gave grounds to suppose that after hormone treatment we can achieve an early recovery of the function of the chain hypophysis-ovary.

Lishman and Inskeep (1991) established that already in the first week after birth may develop a dominant follicle, but it did not ovulate and the performance did not manifest oestral clinical traits. Follicles most often were luteinized and after short-term luteal phase began recovery of the normal ovarian and ovulatory function.

It is proved that different agonists of GnRH gave good results for improvement of sexual cyclic activity of cows after calving birth. The effect depended mainly on the dose and the way of application *(Kunchev et al., 2010).* As the sensitivity of the hypophysis to the performance of GnRH was recovered to the 10th day, then the earliest use was pertinent in the period 10-15 days after calving birth. This procedure led to acceleration of the involution of uterus, shortened the independence period, fertilization with smaller number of insemination and improvement of all reproductive indices in cows.

In her studies about postpartum period in dairy cows, *Deyanova (1995)* established that the concentration of serum progesterone (Pg) and LH increased progressively. Physiological doses of 5 mg GnRH injected in the 8th minute increased the LH content to 4.46 ± 0.59 ng/ml. With increasing the number of days after birth the LH secretion was increased, and the highest values were on the 15th day after calving.

Studies of *Easter (1978)* confirmed the conclusion that the response of the pituitary gland to a large extent was dependent on the amount of steroid hormone in blood. The changes in the sensitivity of the pituitary gland after birth were determined by the activity of the hypothalamus. According to *Fernandes et al., (1978)* this occurred between the 7th and the 10th day after birth. From the absence of a clear reaction in some cows treated with GnRH it is supposed that it may be necessary a longer period for negative effect. The correct answer is probably the natural diversity in the physiology of the course of the follicular phase from the sexual cycle of different animals.

Peters and Lamming (1993) conducted extensive studies on the puerperium of beef cows. They found that the duration of the postpartum period for them was 57.3 days, which was highly influenced by the body weight at birth and by the

season. Cows with better body weight showed oestrus earlier and the duration of postpartum anoestrus in autumn was 35.9 days, and in winter – 60.8 days. The same authors established that plasma concentration of LH from the 1st to 10th day was very low. Probably the refracterness of adenohypophysis in this period was due to the lack of stimulation of the hypothalamus because of reduced or absent pulses in the release of GnRH. It was found that the gonadotropins were secreted in two types – tonic and cyclic *(Di Zerega and Hodgen, 1979).* The cyclic one was characterized by the release of larger amount of hormone, but in shorter intervals of time. Probably this type of secretion was inhibited in the first days after birth. Therefore, more complete determination of the role of hypothalamus in causing the hypogonadotropism which was a main trait in the postpartum period (e.g. hypergonadotropic hypogonadissm), contributed to a large extent to clarify the reasons causing the puerperal anoestrus. After this period, the release of LH started at pulsations (from 1 to 6 till 1 to 4 hours). The secretion of LH at frequency of less than 0.2 pulses per hour was prerequisite for the first ovulation after calving. It was found that the first increase in gonadotropin before ovulation was related to increasing the concentration of progesterone. The reason for this is not known, but it is supposed to be due to luteinization of some later follicle or early release of $PgF_2\alpha$ from the uterus. It is also considered that increasing concentrations of prolactin, leads to more prolonged inactivation of the sexual cycle, especially well manifested in cows with sucking calves. A number of authors associated the duration of the postpartum period with the providing the best possible nutrition of newborn animals. But it is known that sucking is hormone-dependent process, because of which non-endocrinological formulation of postpartum anoestrus is difficult to be accepted. It is supposed that the mechanical irritations of teats and the contact between cow and sucking calf caused release of prolactin and oxyitocin, which suppressed the growth of follicles, ovulation and fertilization and reduced the release of gonadotropins *(Kunchev, 1988).* Since such formulation associated with sucking has profound biological meaning, it should not be neglected. But the complexity of the subject is caused by the fact that in some mammals the influence of sucking is determinative and until it is stopped, the sexual cycle is not recovered. The same is in the pig insufficient! But the situation is different in cows. Many of them are fertilized during lactation and sucking. However, some authors *(Edgerton, 1990; Hanzen, 1996)* established that cows feeding calves showed features of oestrus later in comparison with those that do not feed. Now it is more brought into question the assumption that sucking in cows influences on the lack of ovulation after calving birth. All this imposes the question of postpartum anoestrus to be considered very carefully. For its clarification it is necessary to take into account the role of oxytocin and glucocorticoids. *Goodman et al., (1989)* found that by peptide neurophysin, oxytocin was transferred from supraoptical and paraventricular nucleus of the hypothalamus, where it was synthesized by the posterior pituitary. But it is not clear yet whether the hormone is involved in causing anoestrus in different animal species!? We also know that the amount of glucocorticoids in the peripheral blood circulation can change the

gonadotropin secretion *(Wagner and Li, 1982).* Steroid synthesis of the adrenal cortex was also activated during the process of milking and sucking. In this way the increased concentration of the hormone in blood was preserved during the birth process and also in the early days of postpartum period. What are the actuating mechanisms for tonic inhibition of LH secretion have not been entirely clear yet. Most probably this is due to the reduced sensitivity of the hypophysis to the performance of GnRH, as *Dobson et al., (1987)* establish reduction of the cyclic release of LH after injection of GnRH in cows with increased amount of glucocorticoids in blood.

Conclusion

The physiological dose (100 mcg GnRH) injected intravenously on the 1st day after birth of cows led to increased content of LH in blood circulation.

With increasing the number of days, the secretion of LH from hypophysis was also increased.

The maximum effect occurred after treating with 100 mcg GnRH and it was between the 10th and 15th day after calving.

Ispitivanje dejstva gonadotropin-oslobađajućeg hormona (GnRH) u puerperalnom periodu kod tovnih krava

Ts. Maslev, Ts. Hristova, S. Stoycheva

Rezime

U ovom istraživanju praćen je efekat intravenske injekcije 100 mcg (2 ml) gonadotropin-oslobađajućeg hormona *(Ovarelin, Ceva)* od 1. do 20. dana po telenju krava tovnih rasa, u pogledu pojave prvog estrusa i mogućnosti za njegovu kontrolu. Utvrđeno je da je u prvim minutima intravenske primene *Ovarelina* koncentracija luteinizirajućeg hormona (LH) bila povećana. Uporedo sa povećanjem broja dana povećavala se i količina izlučenog LH. Maksimalan efekat injekcije 100 mcg GnRH postignut je između 10. i 15. dana. Fiziološka doza (100 mcg) GnRH ubrizgana intravenozno 1. dana po teljenja dovodi do povećane koncentracije LH u krvotoku.

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