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## STUDY OF PROTOCOLS FOR SYNCHRONIZATION OF ESTRUS IN BEEF CATTLE

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### *Abstract*

Two protocols for planned synchronization of estrus and ovulation of 53 heifers and 76 cows, of the Aberdeen Angus cattle breed, bred in Bulgaria, have been studied. The experiment was designed to evaluate the pregnancy rates in beef cattle synchronized under four different protocols of synchronization. The first of the protocols for heifers a group of 13 heifers was under Select Synch protocol with extended period of observation and detection of estrus (6-20 days). In the second group (n-40) was implemented standard PRID-Synch protocol. The first synchronization protocol for 31 cows was a modified standard PRID-Synch with a one-day-earlier treatment with PGF $2\alpha$  (i.m.). In the second group (n-45) a standard PRID-Synch protocol was applied. We found that the total pregnancy rate of the first protocol for heifers was 23 %, and regarding the second protocol it was 25%. In cows we established 61.29% for the first group and 75.56% for second.

**Key words:** cow, heifers, synchronization of oestrus, PRID-Synchprotocol.

### INTRODUCTION

The organization of the beef cattle reproduction by means of planned service contributes to a greater economic performance of the herds (Rodgersetal, 2012). The use of appropriate schemes for synchronization of estrus of beef cows is an effective technology which is constantly adapted for the purpose of achieving higher pregnancy rates (Roche and Diskin, 2001; Wiltbank and Pursley, 2014). The elaboration of synchronization of estrus protocols with a fixed-time insemination increases the use of artificial insemination and lowers the expenses related to the monitoring and detection of heat in the beef herds (Bóetal., 2018). The lactation anoestrus in cows leading

to problems upon pregnancy induction within an optimal service period is a considerable problem in the herds with suckling calves (Lambetal., 2010; Gadzirayi, 2010).

The application of estrus synchronization in heifers may contribute to the induction of earlier sexual maturity. According to Damiranetal (2013), the heifers which are calving earlier have higher productivity, remain in the herds longer, and throughout their productive lives, they raise one calf more than the cows which are inseminated at a later age. A higher pregnancy rate is expected if the protocol applied is adapted to achieve follicular synchrony by using GnRH (Thomasetal., 2016).

The duration period of GnRH



administration after prostaglandinane affects the percentage of pregnancies (Bishopetal, 2016), but when compared with heifers, cows exhibit a more expressed heat. Beef cows which are non-cycling during the lactation period display a higher pregnancy rate when they are treated with 300-400 IU eCG during the synchronization protocol (Pessoaetal, 2016).

In order a certain synchronization protocol to be more widely used, it must be cheap and less demanding in terms of time and efforts put, and these can be achieved through a reduction in the number of manipulations (Bóetal., 2014; Whittieretal., 2013). The successful implementation of the modern synchronization methods depends on the complex effect of several factors, the main one being the manner of breeding and the appropriate preparation of the animals prior hormonal treatment (SáFilhoetal., 2010; Ruginosuetal., 2018; Kuzebnyietal., 2018). It is observed that the suckling period and the body condition assessment directly influence the follicular dynamics, the presence of estrus, ovulation and good pregnancy rate in beef cattle (Salesetal., 2011; Uslenghietal., 2014).

The aim of the present study is to research different protocols of synchronization of estrus in Aberdeen Angus beef cows and heifers bred in Bulgaria.

## MATERIALS AND METHODS

The study was carried out in the period 2019-2020. Protocols for synchronization of estrus and ovulation of 53 heifers and 76 cows of the Aberdeen Angus cattle breed, bred in three farms located in Northern Bulgaria, were tested. The animals were divided into four main groups- two heifers and two cows, and two protocols for synchronization of estrus and

ovulation were tested in both animal categories. The first protocol for heifers (PH1) was tested in one group of animals (n-13), and the second one in three groups (n-40). The first protocol for cows included three groups of animals (n-31), and the second one- six groups (n-45).

The choice of heifers to be involved in the experiment was based on an ultrasound examination, reached live weight of 350 kg and an optimal body development. The synchronization included heifers with a normally developed and healthy reproductive system, and the presence of a normal estrus cycle was not taken into account. The cows selection involved evaluation of the body condition (those animals with a mark below 4, based on a 1- to- 9 scale, were excluded from the test) and a healthy reproduction system, at least 45 days after a previous calving. All animals were examined with an ultrasound. Two different protocols for synchronization of estrus and ovulation were elaborated for the two categories- cows and heifers.

From day 32 to day 45 after the artificial insemination, the animals were subjected to an ultrasound examination for the presence of pregnancy. A portable ultrasound machine "WED3000V" with a linear transducer with a frequency of 6.5 MHz was used. The typical reference points for diagnosis of the pregnancy were the presence of an echogenic amniotic fluid in the uterine lumen; the ascertainment of an embryo differentiating itself as a hyperechoic structure; the presence of placentation and so on.

The data are processed via SPSS 21 with models of the following statistical type:

$$Y_{ij} = \mu + K_i + PK_{ij} + e_{ij} \text{ (Model 1),}$$

$$Y_{klm} = \mu + P_k + e_{klm} \text{ (Model 2),}$$

where  $Y(\dots)$ - observation vector;  $\mu$ -



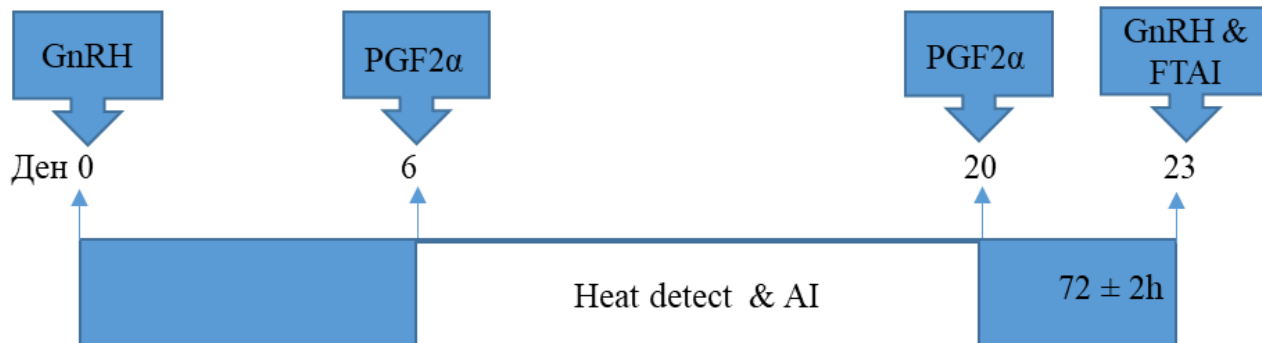
total average constant; P and K are fixed effects of respectively the i- age category (cows, heifers) and k- protocol; PK is the random effect of the j- protocol within the scope of the i-category; e(...) – residuals.

## RESULTS AND DISCUSSION

Two protocols for synchronization of estrus and ovulation of heifers (Protocol for heifers 1 (PH1) (n-13) and Protocol for heifers 2 (PH2) (n-40)) were elaborated and tested for the purposes of the present study.

Figure 1 displays PH1 which may be classified as a SelectSynch protocol with an extended monitoring and ascertainment of estrus period (from 6<sup>th</sup> to 20<sup>th</sup> day). On day 0, each animal was intramuscularly injected with 100 µg GnRH (gonadorelin) or 2ml OVARELIN®. On day 6, ENZAPROST T- single administration of 25mg dinoprost

(PGF2α) (5ml of the product per animal) was intramuscularly injected. From the sixth until the twentieth day, the animals were monitored for detection of heat and the heifers exhibiting typical signs of estrus were artificially inseminated. On the twentieth day after the start of the protocol all animals which had not displayed estrus yet were injected with PGF2α again, and 72 hours later they were inseminated (FTAI). VITAMIN AD<sub>3</sub>E (Active substances: Vitamin A - 1,500,000 IU/100ml; Cholecalciferol (VitaminD<sub>3</sub>) – 2,000,000 IU/100 m; AlphaTocopherylAcetate (VitaminE) - 1.0g/100 ml) was additionally injected on the 6th and the 20th day. 32 days after the insemination, all heifers part of the protocol were subjected to an ultrasound examination (US) for the presence of pregnancy.



**Fig. 1.** Protocol 1 for synchronization by heat detection and artificial insemination and fixed time for artificial insemination in beef heifers of the Aberdeen Angus cattle breed

PH2 is a standard protocol for synchronization of estrus with the use of intra vaginal devices PRID delta (Fig. 2). On day 0, each animal was injected 100 µg GnRH

(gonadorelin) (2 ml OVARELIN® per animal) and inserted PRID delta progesterone-releasing vaginal devices for cows containing 1.55g progesterone each.



**Fig. 2.** Protocol 2 for synchronization of estrus and ovulation with a fixed time of artificial insemination in beef heifers of the Aberdeen Angus cattle breed

The removal of the vaginal progesterone devices was performed on the 7<sup>th</sup> day after the initiation of the protocol and each animal was intramuscularly injected with Folligon (MSD Animal Health) 500 UI and ENZAPROST T (Dinoprost such as Trometamol, 5 mg/ml) - a single administration of 25 mg dinoprost (5 ml dose of the product) per animal. The artificial insemination was performed at a fixed time on the 72<sup>nd</sup> hour after the removal of the progesterone devices in combination with an intramuscular injection of 100 µg GnRH (gonadorelin), per animal. On day 32 all animals from the heifers test group were subject to an ultrasound examination for the ascertainment of pregnancy.

Both protocols tested in the cows were based on intra vaginal progesterone devices with the key difference between them being the time of prostaglandin application. Day 0 which is the day of application of the intra vaginal

devices is considered the start of the first protocol for cows (PC1) tested by us. The devices remained in the vagina for seven days (Fig. 3). On day 6 the animals were intramuscularly injected with PGF2α ((Oestrophan) (Cloprostenol 250mg/1ml)), 2 ml per cow. Upon removal of the devices on day 7, all animals were injected with 500 IU Equine serum gonadotrophin (eCG) corresponding to 2 ml of the solution. The planned artificial insemination was performed at a fixed time from the 52<sup>nd</sup> to 56<sup>th</sup> hour after the removal of the progesterone devices. Upon insemination, each animal was intramuscularly injected with 100 µg GnRH (gonadorelin)/(2 ml) OVARELIN®, per animal. This protocol was applied in two farms in the village of Lesidren- 20 cows and in the village of Kovatchevitsa- 11 cows of the Aberdeen Angus cattle breed.



**Fig. 3.** Protocol 1 for synchronization of estrus and ovulation with a fixed time of artificial insemination in beef cows from the Aberdeen Angus cattle breed

Figure 4 displays Protocol 2 (PC2) which is also PRID delta based. PC2 is applied to 45 cows of the Aberdeen Angus cattle breed. On day 0, the animals were intramuscularly injected with 100 µg GnRH (gonadorelin)/(2 ml) OVARELIN® and PRID delta progesterone releasing devices for cows, containing 1.55 g progesterone each were inserted. The removal of the intra vaginal progesterone devices was performed on the 7<sup>th</sup> day after the initiation of the protocol when the animals were also intramuscularly injected

with Folligon (MSD Animal Health) 500 UI and ENZAPROST T (Dinoprost such as Trometamol, 5 mg/ml) - 25 mg dinoprost (5 ml dose of the product per animal). The artificial insemination was executed at a fixed time at the 72<sup>nd</sup> hour after the removal of the progesterone devices in combination with an intramuscular injection of 100 µg GnRH (gonadorelin)/ (2 ml) OVARELIN® per animal. The protocol was implemented on 45 cows of the Aberdeen Angus cattle breed in the village of Doktor Yosifovo.



**Fig. 4.** Protocol 2 for Synchronization of estrus and ovulation with a fixed time of artificial insemination in beef cows from the Aberdeen Angus cattle breed

Table 1 shows the results of the application of the protocols for synchronization of estrus in the cows and heifers subject to our study.

The synchronization schemes for the heifers are based on different approaches: with or without the use of PRID devices; partially inseminated animals with an estrus detection or



with FTAL.

According to Perry (2016) the protocols for synchronization of estrus which include progestin may induce estrus cycle in non-cycling heifers. During and after progesterone treatment it is expected that the secretion of critical hormones such as LH will increase which in turn will stimulate the follicle growth in the ovaries, and also the ovulation which starts spontaneously or as a response of exogenous factors (Day and Nogueira, 2013). However, the differences between the pregnancy rates in both variants are minimal, in PH1 the pregnancy rate has been 23%, and

in PH2- 25% ( $P < 0.001$ ). Low pregnancy rates upon synchronization of heifers has been also observed by other authors (Butler et al., 2011; Colazo and Mapletoft, 2014).

The pregnancy rates of the cows subject to our study were considerably higher- 69, 7% than that of the heifers- 24, 5%, and the differences are reliable ( $P < 0.001$ ). It is noticeable in the two schemes for synchronization of cows examined that in PC2, in which the insemination was carried out at the 72<sup>nd</sup> hour, the pregnancy rate was higher- 75, 6% than that in PC1- 61, 29%, ( $P < 0.001$ ).

**Table 1.** Pregnancy rates upon application of schemes for synchronization of estrus and ovulation in cows and heifers.

Protocol	N	Pregnant, %	±SE
PH1	13	23,08	12,52
PH2	40	25,00	7,138
PC1	31	61,29	8,108
PC2	45	75,56	6,729

The studies on the success rates of the protocols for synchronization for the beef cattle are quite controversial. Malik et al., (2012) reports pregnancy rates of 23,3%- 37.5 % in the different groups. In another study, Bóetal., (2018) announce that upon use of the classical protocols for synchronization of estrus and ovulation in the beef cattle herds in South America, the pregnancy rates are from 40 to 60%.

**Table 2.** Influence of the protocol for synchronization and the category on the pregnancy rates in cows and heifers.

Model	Factor	F	Sig.
Model 1 (cows and heifers)	Protocol*Category	11,05	0,000
Model 2 (heifers)	Protocol	0,019	0,891
Model 2 (cows)	Protocol	1,764	0,188



Upon examining the factors affecting the efficiency of the protocols for synchronization tested, we ascertained reliable influence ( $P < 0.001$ ) of the protocol within the scope of the category. Upon use of a single factor analysis with a fixed effect of the protocol, which is accounted in both of the categories, there is no reliable influence observed regarding the percentage of pregnancies after the synchronization. (Table 2).

### CONCLUSION

Two protocols for synchronization of estrus and ovulation were tested in cows and heifers of the Aberdeen Angus cattle breed. The cows displayed higher pregnancy rates – 69.7% than the heifers- 24, 5%, and the differences are reliable ( $P < 0.001$ ).

The differences between the pregnancy rates in the tested protocols for heifers are negligible- the percentage of pregnant animals was 23% (Protocol 1 for heifers) and 25% (Protocol 2 for heifers).

The two tested protocols for cows display higher pregnancy rates- 61,29 % (Protocol 1 for cows) and 75, 6% (Protocol 2 for cows).

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