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STUDY OF PROTOCOLS FOR SYNCHRONIZATION OF ESTRUS IN HEIFERS WITH SEX-SORTED SEMEN

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Abstract

Two protocols for planned synchronization and resynchronization of estrus and ovulation of heifers (n-25), aged between 11 and 18 months, of the Aberdeen Angus breed, bred in Bulgaria, have been studied. An experiment was designed to evaluate pregnancy rates in beef heifers synchronized in two different protocols with consecutive synchronization and resynchronization. The first protocol is a combination of two standard and may in principle be divided into two consecutive stages. In the first stage, a group of 11 heifers are under Select Synch protocol extended period of observation and detection of estrus (6-20 days). In the second stage, after 32 days and ultrasound pregnancy detection, standard PRID-Synch protocol and timed artificial insemination, are applied for the resynchronization. In second protocol standard PRID-Synch protocol and timed artificial insemination are applied for synchronization and resynchronization of group of 14 heifers with sex-sorted semen. We found that the total pregnancy rate for the first protocol is 32.39 ± 10.54 % and for the second protocol is 23.8 ± 8.92 %.

Key words*: estrus synchronization; heifers; sex-sorted semen*

INTRODUCTION

Bulgarian beef cattle production is a subsector which has been paid more and more attention. Since the beginning of the century there has been a transition from dairy to meat cattle breading which is becoming even more intensive in the recent years. When compared to 2016, in 2017 the livestock farms in the country mark a general decline by 15.1% but the number of beef cows increases by 12.5% (MAFF, 2018) Beef cattle are monoproductive, and the major factor determining the production technology efficiency is the organization and management of the reproductive process. López-Paredes et al. (2018) have ascertained that the reduction of the first calving age leads to a cut in the heifers feeding expenses, decrease in the production costs and increase of the profits. According to Damiran et al. (2018), the heifers which calve earlier have higher productivity, remain in the herds longer, and throughout their productive live, they raise one calf more than the cows which are inseminated at a later stage.

The approaches related to the induction of earlier sexual development in heifers are concentrated around exogenous treatment with the hormone progesterone either individually or in combination with others such as oestradiol, GnRH or equine serum gonadotropin (eCG) which all bolster ovulation induction. These induction protocols are based on the presumption that the progesterone may reprogramme the immature reproduction system control in such a way that it may cause maturation and normal functioning. It is expected that during and after progesterone treatment, the secretion of hormones such as LH will increase and this will stimulate the follicles growth in the ovaries as well as the ovulation which occurs spontaneously or as a response of exogenous factors (Day and Nogueira, 2013).

The estrus synchronization has several major advantages- it can affect the length of the calving interval, it also reduces the necessity of constant monitoring for estrus displays in cows, facilitates the insemination campaign planning, equalized calves groups production, and so on. Application of artificial insemination in extensive beef cattle breeding may be eased through the use of effective protocols for synchronization of the estrus and the ovulation with a subsequent fixed time artificial insemination (FTAI) (Echternkamp and Thallman, 2011). According to Gabriel et al. (2016), the fixed-time artificial insemination is considered to be the most useful method for

increasing the number of inseminated cows in the herds.

The aim of our study is to examine the effectiveness of different protocols for synchronization and resynchronization of the estrus of Aberdeen Angus heifers bred in Bulgaria inseminated with a sex-sorted semen.

MATERIALS AND METHODS

The study was carried out in 2019. Protocols for planned synchronization and resynchronization of estrus and ovulation of heifers of the Aberdeen Angus Cattle breed (n-25), bred in „THRACE ANGUS FARM'' LLC, Montana region have been studied. The location of the farm is suitable for breeding of a specialized beef cattle breed such as the Aberdeen Angus. The manner of breeding in the farm is free in groups on deep litter bedding which is periodically cleaned. The buildings used are semi-opened, and the premises- zoned. Each cattle group and category has a yard for walks during the winter months. In the summer, the main categories, with the exception of the calves for fattening, are on the pastures. The farm is a single-sector and non-specialized one with a full reproduction cycle.

The heifers are aged between 11 and 18 months and are divided into two groups, depending on the synchronization protocol, following the method of analogues. The choice of heifers for the experiment in the groups is based on an ultrasound examination, attained minimum live weight of 350 kg, and an optimal physical development. The heifers included in the trial synchronization have a normally developed and healthy reproductive system but the presence of normal estrus cycle is not taken into consideration.

Two different protocols have been elaborated for the purpose of inducing estrus and ovulation synchronization and resynchronization.

The heifers were inseminated with a sex-sorted semen (for conceiving of female calves only). On day 32 after the insemination, the heifers 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 for the performance of the ultrasound check-ups. 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 as a hyperechoic structure; the presence of placentation and so on. During the manipulations the animals are fixed in a crush and all requirements and conditions for carrying out the procedure were met.

Two parameter groups were examined for the assessment of the two synchronization protocols. The first group includes financial assessment which is actually the valuation of a single synchronization and an average cost of a successful pregnancy. The financial value includes only the price of the veterinary and medical products, and the price of the semen and the work performed are not taken into account as they are largely fluctuating parameters. The average value of a successful pregnancy- Average pregnancy value (APV) is calculated with the following formula: APV= NS (number synchronization) * POS (price of one synchronization)/ NB (number of established pregnancies).

The second group of features analyzed- commercial parameters include the number of animal manipulations and protocol duration.

The pregnancy rate data are processed statistically via the specialized software SPSS21, IBM.

RESULTS AND DISCUSSIONS

Two protocols for synchronization and resynchronization of estrus and ovulation of heifers of the Aberdeen Angus Cattle breed with a sex-sorted semen were elaborated and tested for the purposes of the present study.

Figure 1 displays Protocol 1 (Pr1). The protocol is a combination of two standard synchronization schemes and can be generally divided into two consecutive stages. During the first stage a group of 11 heifers is subject to Select Synch protocol with an extended monitoring of estrus period (from 6th to 20th day). On day 0, each animal is intramuscularly injected 100 μg GnRH (gonadorelin) or 2ml OVARELIN®, Ceva Sante Animale France. On the sixth day 25 mg of PGF2α (ENZAPROST T, Ceva Sante Animale, France) one application of 5 ml of the product per animal was intramuscularly injected. From the sixth until

the twentieth day the animals were monitored for the ascertainment of heat and the heifers exhibiting typical signs of estrus are inseminated. On the twentieth day after the start of the protocol all animals which have not displayed estrus yet are injected PGF2α again and 72 hours later they are inseminated (FTAI). Thirty-second days after the artificial insemination (day 55), all heifers which are part of the protocol are examined with an ultrasound (US) and those which are not pregnant are subject to resynchronization (Second stage of the protocol). A standard PRID- Synch protocol and fixed-time artificial synchronization (FTAI) is applied during resynchronization. On day 55, each animal is injected 100 µg GnRH and progesterone-releasing intravaginal device (PRID delta, Ceva Animal Health) are inserted for heifers each containing 1.55g progesterone. The progesterone-releasing intravaginal devices are removed after they have remained in the vagina for seven days on day 62-nd, and at the same time the animals are intramuscularly injected with eCG 500 UI (Folligon, MSD Animal Health) and 25 mg of PGF2α (a single 5ml dose of the product per animal). The artificial insemination is performed at a fixed time on the 72 hour after the removal of the progesterone-releasing devices in combination with an intramuscular injection of 100 µg GnRH per animal.

VITAMIN AD$_3$E (Active substances: Vitamin A - 1,500,000 IU/100ml; Cholecalciferol (VitaminD$_3$) – 2,000,000 IU/100 ml; Alpha Tocopheryl Acetate (Vitamin E) - 1.0g/100 ml) is additionally injected on the 6th and the 20th day.

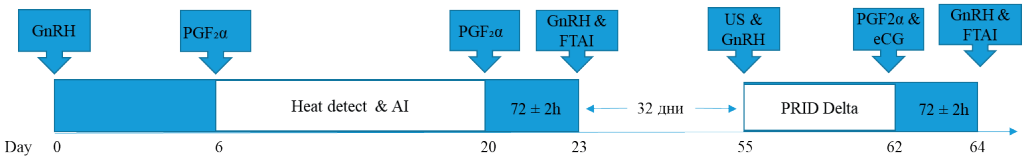

Figure 1. Protocol 1 for Synchronization and Resynchronization by Heat detect and AI and FTAI in beef heifers from the Aberdeen Angus Cattle breed

Figure 2 shows Protocol 2 (Pr 2) for synchronization and resynchronization performed through a two-time consecutive application of a standard protocol for synchronization of estrus with the help of intravaginal devices PRID delta. On day 0, each animal is injected 100 µg GnRH and inserted a PRID delta progesterone-releasing devices containing 1.55g progesterone each.

The vaginal progesterone-releasing devices are removed after they have remained in the vagina for seven days, and at the same time the animals are intramuscularly injected with eCG 500 IU (Folligon, MSD Animal Health) and PGF2α (ENZAPROST T, Dinoprost such as Trometamol, 5 mg/ml) -a single administration of 25 mg dinoprost or 5 ml dose of the product per animal. The artificial insemination is performed at a fixed time on the 72-nd hour after the removal of the PRID delta in combination with an intramuscular injection of 100 µg GnRH per animal.

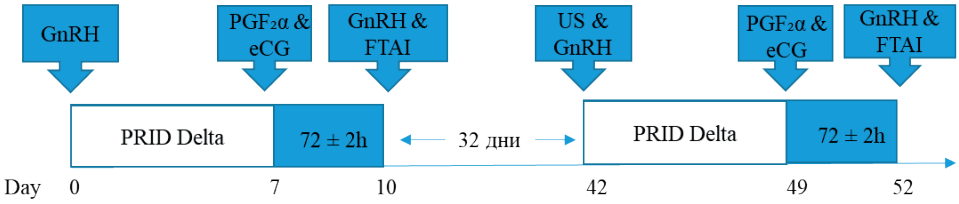

Figure 2. Protocol 2 for Synchronization and resynchronization of estrus and FTAI in beef heifers from the Aberdeen Angus Cattle breed

On the 32nd day after the artificial insemination, all heifers which are part of the protocol are examined with an ultrasound (US) for the presence of pregnancy. Those which are not diagnosed like pregnant undergo the same synchronization regimen again, i.e. resynchronization or second stage of protocol.

Table 1 displays the results of the protocol for synchronization of heifers application. According to Perry (2016), the estrus synchronization protocols which include progestin may induce estrus cycle in non-cycling heifers. However, we have ascertained that during the first stage of Pr 1, in which progesterone devices are not used, the pregnancy rate of heifers was almost two times higher- 27.27 % than it was during the second scheme (14.29 %) in which such devices were used in both stages of the synchronization. The difference during the second stage is negligible- 37.5 % and 33.3 % for Pr 1 and Pr 2, respectively. The total AI pregnancy rate indicated in our experiment is 31.58 % and 23.8 % in Pr 1 and Pr 2, respectively and have not been ascertained significant differences. On one hand, the low pregnancy rates might be explained by the inability the follicular waves in heifers to be fully synchronized. On the other hand, they might be due to the lower pregnancy rates which are expected upon sex-sorted semen insemination.

Usually, an average pregnancy rate expected in programs for synchronization of estrus is 40-60 % although the percentage of animals which exhibit estrus is bigger.

Table 1. Pregnancy rates upon testing of schemes for synchronization of estrus of heifers of the Aberdeen Angus Cattle breed

Stages	Protocol 1				Protocol 2			
	N	Pregnant, number	Pregnancies per artificial insemination (P/AI), %	±SE	N	Pregnant, number	Pregnancies per artificial insemination (P/AI), %	±SE
Stage 1	11	3	27.27	13.68	14	2	14.29	12.12
Stage 2	8	3	37.50	16.04	12	4	33.33	13.10
Total	19	6	32.39	10.54	26	6	23.81	8.923

Similarly to us, upon testing synchronization protocols for heifers, Butler et al. (2011) ascertain a pregnancy rate of about 30%. According to plenty of authors, the sex-sorted semen artificial insemination leads to a lower pregnancy rate in heifers which are subject to synchronization (Mallory et al., 2013; Thomas et al., 2014; Crites et al., 2018). According to Thomas et al. (2017), when using of sex-sorted semen, it is necessary maximum heat to be reached prior application of artificial insemination because the sex-sorted semen has a smaller number of spermatozoa per dose and a lower survival rate after thawing. In this respect, the explanation of the two times higher results of stage one in Pr 1 may be found not in the presence or the lack of progestins but in the insemination which is performed after heat detection.

In the process of studying some basic parameters related to the value, complexity and labour intensity of the protocols compared, we have ascertained that in Protocol 1 the number of the manipulations carried out and the time consumed for their realization are more than those in Protocol 2 which comes to prove that Protocol 1 is more complex and labour intensive (Table 2).

Table 2. Basic parameters in trial of schemes for synchronization of estrus

Parameters	Scheme	Scheme 1	Scheme 2
Financial parameters	Cost of veterinary and medical products , €	30	40
	Cost per pregnancy, €	95	173
Marketing parameters	Number of manipulations of animals	7	6
	Duration, days	67	53

The larger number of manipulations which are performed is connected with more labour and also leads to more stress for the animals themselves. On the other hand, the cost of the necessary materials per pregnancy is considerably lower and the success rate of Protocol 1 - higher.

CONCLUSIONS

In synchronization of the estrus of heifers of the Aberdeen Angus Cattle breed and subsequent sex-sorted semen insemination, higher results (pregnancy rate of 31.58%) are achieved in the protocol in which a scheme with an extended period of estrus detection without the use of progesterone-releasing intravaginal devices during the first stage is applied, while the pregnancy rate after the application of protocol for standard scheme synchronization and resynchronization with progesterone-releasing devices and FTAI is 23.8%.

In synchronization of the estrus of beef heifers inseminated with a sex-sorted semen, the protocol in which the insemination is after estrus detection is more suitable as it compensate the low sperm count and reduced viability, although it is more laborious and prolonged in time.

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