Comparative studies of inducing estrus in suffolk breed sheep, as the choice of the optimal receptor synchronization scheme, in vivo embryotransfer protocol

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Abstract
Reproductive biotechnologies in sheep, are important tools for genetic improvement because they enable to increase the gene frequency of individuals with superior genotype. The techniques for AI and ET of sheep and goats differ from those normally used in other farm animals mainly due to their anatomical peculiarities. Embryo transfer biotechnology (ET) is applied to females of superior genotype (like the Suffolk race) and aims to increase the frequency of their genes by increasing their progeny. ET allows the transference of embryos from superior females (donors) to matrices with low genetic value (recipients). In this study we followed the protocol for the induction and stimulation of estrus in Suffolk sheep. Also, the reactivity of the sheep to different schemes for the treatment of estrus synchronization was followed. The purpose was to obtain, test and recommend a certain treatment for the synchronization of embryos donors and receptors, part of ET. Therefore, a number of 60 Suffolk sheep were treated with different hormones, divided into 3 groups of 20 each. Group A received P12-PGF; group B, P12-PGF-GnRH; and group C, P12-PGF-PMSG. Following treatment, the sheep were monitored on days 1-3 for signs of estrus, which were observed in most sheep. Thus: at Gr A, 16/20 (75%), at Gr.B, 18/20 (90%) and at Gr C, 20/20 (100%). Regarding ovarian response and synchronization of estrus for the anticipation of ovulation, (after the time of onset), the most favorable response was obtained in group B. Following treatment with P12-PGF-GnRH, estrus was manifested on the second day. For the synchronization of embryo receptors in sheep of the Suffolck breed, we recommend scheme B, because it synchronizes the ovulation in most sheep.
Keywords*: sheep Suffolk, estrus, synchronization, MOET*

Introduction

Reproductive activity in sheep is characterized by a seasonality influenced by several factors such as photoperiod, latitude, temperature, nutrition and breed (fig no.1).
Estrus control is a management tool helping to reduce time and effort involved with estrus detection by clustering individual estrous periods and, if combined with ovulation induction, permit timed AI.

The ewes are seasonal breeders and exhibit estrus only during few months of the year. Methods of induction and synchronisation of estrus can be divided into 2 main groups: hormonal and non-hormonal methods. The hormonal methods are intended to shorten the life span of an existing corpus luteum by administering of an exogenous luteolysin, or to simulate the corpus luteum function by administering progestogens for many 9 to 19 days. Alternatively, esrtus synchronisation may be achieved by manipulation of both follicular and luteal phase through GnRH in combination with PGF2α.

The induction of estrus in ewes during seasonal anoestrous was accomplished with intravaginal sponges impregnated with progestagens, medroxyprogesterone acetate (MAP) (Evans et al., 2004), inserted from 12 to 14 days. Subsequently, equine chorionic gonadotropin (eCG) was administered (Zeleke et al., 2005). Nevertheless, the fertility of anestrous ewes treated with progestagen ranged from 22 to 70% (Evans et al., 2001).

Long term progestagen estrus synchronization protocols can affect follicular dynamics and fertility of ewes (Viñoles et al., 2001; Diskin et al., 2002). Initially, a supraluteal effect is expected, which means that an increase in follicular renewal may occur. In the end, however, a subluteal effect may happen and decrease the speed of follicular renewal (Hamra et al., 1986). The use of progestagen during a short time would be an alternative solution because it could improve estrus synchronization in ewes (Husein et al., 2007).

A combination of progestagen with eCG was used in ewes for 6 days and resulted in good fecundity. It was enough to induce and synchronize estrus in approximately 90% of the animals (Ataman et al., 2006).

The Suffolks are a British breed of domestic sheep. They are polled, and have black open faces along with black legs and white-woolled bodies. Suffolks are considered a large breed of sheep, their large frame and muscular bodies make them an ideal breed for meat production, however; they are also good for wool production as well. Suffolk rams are commonly used as a terminal sire on cross-bred ewes due to their ability to produce off-spring with excellent growth and carcass traits.

Important terms about ewe reproduction:

Estrus (Heat) or Estrous Period. Period of time when ewe or doe will receive the male, ovulate, and egg can be fertilized. During 20-42 h long in small ruminants. Signs include standing, turns head towards male, raises dock or flags tail, bleating. Ovulation occurs approximately 30 h into heat.

The productivity of indigenous sheep is low, due to poor genetic merit (Alam et al.,2001), poor nutrition (Alam et al., 2006), and weak management. Multiple Ovulation and Embryo Transfer (MOET) is well accepted and applied worldwide to speed up genetic gain through production of large number of lambs, reducing generation interval and utilization of superior dams (Bari et al., 2003; Menchaca et al., 2009,). The MOET program, followed by direct transfer into recipients, can reduce the cost of embryo transfer, if the estrus of donors and receptors is concomitant and the time of ovulation is synchronized at the same time (S Ghosh. 2017).

Biotechnology of embryo transfer including fresh and conserved embryos is limited in small ruminants compared with large ruminants, but the *In vivo* EmbryoTransfer program, can reduce costs and increase the use of this technique in ewes (Zamfirescu S. 2009).

Materials and Methods

The present study was conducted during the weeks of September (autumn) at a private farm in Bacau, in the N-Eastern region of Romania. This period is accepted as it represents the onset of the natural breeding season of smol ruminants in region.

Selection of the ewes

A total of 60 Suffolk ewes, aged 2.5–5 years-old were used in this study. One month before beginning of the study, each goat was subjected to general physical examination, and the goats were dewormed orally with anthelmintic.

The ewes were maintained graze on natural pasture from 06:30 to 18:00 h and kept in pens during nights. Water and a mineral supplement were available ad libitum. The management of the goats did not change throughout the experimental period.

The ewess were randomly divided into three equal groups (20 each), and were registered by attaching numbers on their ears.

Oestrus synchronization of the ewes.

Therefore, a number of 60 Suffolk sheep were treated with different hormones, divided into 3 groups of 20 each. Group A received P12-PGF; group B, P12-PGF-GnRH; and group C, P12-PGF-PMSG, (fig no 3).

All groups received an intravaginal sponge (Chronogest, Intervet) containing 30 mg fluorogestone acetate (FGA) for 12 days.

To the Group A, after 11 days (one day before the sponge is removed) a dose of cloprostenol equivalent to 125 µg prostaglandin F2α was administered (Estrumate 0,6 cc.).

To the Group B, after 11 days a dose of prostaglandin F2α was administered (Estrumate 0,6 cc.), and on day 12 received 10.5 µg busereline acetate (GnRH, Receptal, Intervet).

To the Group C, after PGF2α (day11) and on day 12, ewes received 200 IU Pregnant Mare Serum Gonadotrophin (PMSG; Folligon®, Intervet).

Detection of oestrus

The ewes were kept with teaser rams, and observed for signs of oestrus twice a day for 60 minutes in each observation from 48 hours after second injection of prostaglandin F2α (fig no 4).

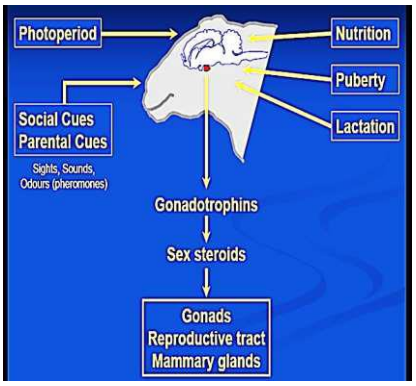

Fig no 1.
Factors that condition the breeding season in sheep, (Zeleke Mekuriaw- 2104)

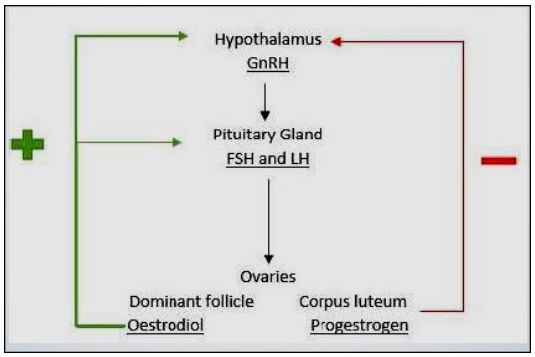

Fig no 2.
Chronogest CR Intravaginal Sponges, and its mechanism of action
Chronogest sponges are impregnated with 20mg flugestone acetate, a progestogen.

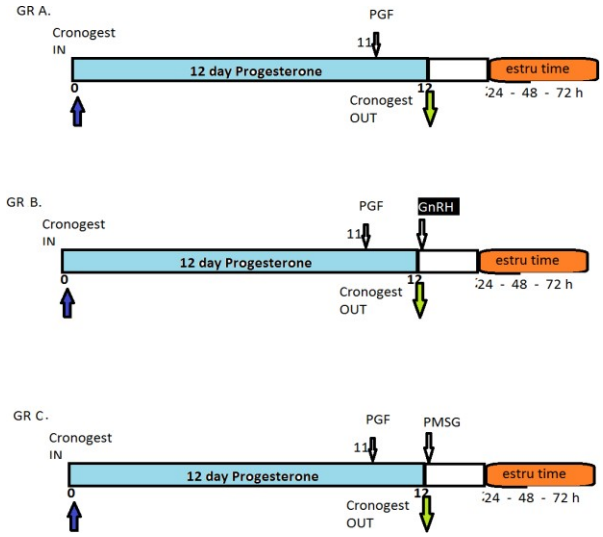

Fig No 3 Hormonal treatment schemes applied to sheep
Gr.A: P12-PGF;
Gr.B: P12-PGF-GnRH;
Gr.C: P12-PGF-PMSG.

Fig No 4 Suffolk breed sheep in the heat, the preovulatory moment

Rezult and discutions

In this study we followed the protocol for the induction and stimulation of estrus in Suffolk sheep. Also, the reactivity of the sheep to different schemes for the treatment of estrus synchronization was followed. The purpose was to obtain, test and recommend a certain treatment for the synchronization of embryos donors and receptors, part of ET.

An effective estrus synchronization regimen is expected to synchronize the estrus of the treated animals within a 12 to 24-h period, stimulate high rates of oestrus and ovulatory response, and enable the achievement of a high pregnancy rate with artificial insemination. The most common methods used for the hormonal control of the oestrus cycle in ewes include the establishment of an artificial corpus luteum (CL) function through the administration of progestogens for a certain time period, the stimulation of luteolysis by means of the administration of luteolytic agents, and the synchronization of ovulation by means of the combined administration of gonadotropin-releasing hormone (GnRH).

In this method, the function of the corpus luteum is simulated by application of progesterone or one of its analogous compounds. The release of gonadotropins is inhibited by progesterone, and, hence, the ovulation is also inhibited until progesterone is removed (fig no2). If progesterone is applied for a group of females and withdrawn simultaneously, this will synchronize the estrus and ovulation in this group.

Progesterone was initially delivered for a period equal to the length of the natural luteal phase. There are various administration time protocols such as: Long-term progesterone treatments (18 to 21 days), and Short-term progesterone treatments (7 to 12 days). Therefore, it is crucial to include a luteolytic agent in combination with short-term progesterone treatments in order to get rid of any natural corpus luteum. This technique is applicable for cycling and acycling ewes during the breeding and non-breeding season, but in this case, ovulation induction is required e.g. administration of 200 to 400 IU eCG.

An injection of PGF2α, or one of its analogs, during the mid-luteal phase of the estrus cycle can induce a premature CL regression and ewes, therefore, can be expected to exhibit estrus symptoms approximately 50 hours later.

In our study, following treatment, the sheep were monitored on days 1-3 for signs of estrus, which were observed in most sheep. Thus: at Gr A, 16/20 (75%), at Gr.B, 18/20 (90%) and at Gr C, 20/20 (100%).

The ovarian response, and the occurrence of estrus in group A, was 75%, the response to treatment is favorable, but it does not guarantee the ovulation. Ovulation occurs only uncontrolled by the discharge of endogenous LH.

In group B, the percentage of estrus grouping was better than 90%, compared to the previous one. The response to treatment is perfect. GnRH administration on day 12 generated a pituitary gonadotropin discharge. We argue that endogenous secretion was mainly induced by LH, which guarantees the ovulation group.

In group C, the percentage of estrus grouping was maximum 100%. The response to treatment is also perfect. PMSG administration on day 12 induced ovarian follicle growth and maturation, and estrus synchronization. However, the treatment does not guarantee ovulation and synchronization in all sheep.

Regarding ovarian response and synchronization of estrus for the anticipation of ovulation, (after the time of onset), the most favorable response was obtained in group B. Following treatment with P12-PGF-GnRH, estrus was manifested on the second day (40-50 h). For the synchronization of embryo donors and receptors in sheep of the Suffolck breed, we recommend scheme B, because it synchronizes the ovulation in most sheep.

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