Efficiency of prostaglandins and progestogens in synchronization and stimulation of estrus in recipient goats

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Abstract. Intravaginal implants containing 40 mg of progesterone for synchronization and stimulation schemes of estrus in recipient goats, with a period of exposure to the animal’s body of 6 and 12 days in combination with fertadine and folligon at different reproduction periods during the year, allows to reduce time of estrus onset in animals up to 30 hours or 2.5 times compared to goats treated only with prostaglandins, while inducing estrus in 100.0% of goats with embryo engraftment in recipients of 56.7% on the 40th day of diagnosis and 50.0% upon animals birth with litter yield of 146.7%.

1. Introduction
The rapid development of biotechnology has radically changed the possibilities and efficiency of breeding. One of the key points for accelerated reproduction of valuable animal genotypes is development of fundamental applied foundations of biotechnological methods aimed, first of all, at the maximum use of reproductive abilities of embryo donor dams. So, the number of offspring from one goat in its entire life is from 3 to 6 goatlings. The offspring of genetically valuable producing goats at artificial insemination can number up to several tens of thousands of animals. Meanwhile, the biological abilities of reproduction of dams are great, the ovaries of newborn goats contain up to 50-70 thousand potential oocytes. For a fuller use of this enormous genetic potential, the biotechnological method – embryo transplantation – serves as an indispensable tool.

Successful transplantation of the extracted biomaterial to recipients is one of the conditions for efficient work on production of transgenic animals. Zygote transplantation includes several stages, including hormonal preparation of recipients or choosing them in spontaneous estrus is a prerequisite for preparation of animals with determination of estrus signs during induced and natural estrus. Efficiency of work on synchronization-and-stimulation of estrus in animals is determined by the choice of hormonal medicines, treatment schemes and methods, seasonality, stage of estrous cycle, age of goats, etc. [1-4].

So, Ebert K.M. et al. [1] obtained 50% acceptability was obtained, established by the ultrasound method on days 45 and 55 after transplantation of goat biomaterial synchronized with ear implants with a norgestomet (Synhromate-B, 6 mg). On the 7-9th day, the recipients were injected with
prostaglandin, and on the 13th – 400-750 IU of PMSA, depending on the season of the year. As a result of transplantation, 29 goatlings were obtained.

According to other data [2-3], stimulation-and-synchronization of the recipient cycle with an analogue of prostaglandin F₂α with a simultaneous injection of 400 IU of PMSA contributed to 34.8% of embryo acceptability. At the same time, use of pessaries for 13 days and the injection of 400 IU PMSA 12 hours prior to the removal of the sponges led to pregnancy in 40.0% of the animals, but no offspring were obtained.

According to Freitas V.J.F. et al. [4], use of intravaginal sponges impregnated with 45 mg FGA (Synchro-part, Sanofi, France) for 11 days with an injection on day 9 of 50 μg of cloprostenol and 200 IU eCG (Syncho-part, Sanofi) made it possible to identify estrus in 94.1% of recipients with 2 to 13 ovulations obtained (5.3 per recipient average), and the average time interval from the end of treatment to manifestation of estrus signs was 22 hours.

The most important element of obtaining a high acceptability of biomaterial in recipient animals is the accuracy of synchronization of the donor’s and recipient’s estrus. It is believed that the maximum correspondence between the hormonal background and the biochemical status of animals contributes to recognition by the recipient’s body of the signal emanating from the embryo placed in the oviduct or uterus, followed by physiologically determined processes of embryo nidation, normal development and pregnancy process. In order to exclude a number of factors causing decrease in efficiency in terms of survival rate, the selection of recipients is based on the presence of a pronounced estrus and physiological state of ovaries. [4-6].

Armstrong D.T. et al. in his research [7] obtained no reliable result on acceptability of goat embryos during estrus synchronization in donors and recipients ± 1 day: 50.0-, 60.0- and 57.2% pregnancies in recipients were diagnosed with estrus in 1 day, simultaneously and on the next day with the donor, respectively.

According to Baldassare H. et al. [8], asynchrony of ±12 hours of estrus in donors and recipients influenced the pregnancy rate of the latter (-12=55%, 0=43%, +12=50%). The difference in this indicator between the groups of animals made 5-12%. Goldman I.L. et al. [9] and Ernst L.K. et al. [10] proposed to use a conveyor system for transplanting biomaterial, involving the use of donors as recipients, since a high degree of synchronization of estrus cycles and physiological state of animals is achieved here, which makes it possible to obtain from 40 to 56% of pregnant sheep. The aim of research was to determine the efficiency of various hormonal agents and their complexes during synchronization-and-stimulation of estrus in recipient goats and their effect on acceptability and offspring after goating.

2. Research methods
The research was carried out at the Biotechnological Research and Experimental Production of Animal Transgenesis in the Laboratory for Reproduction, Embryo Transplantation and Animal Transgenesis of the Republican Unitary Enterprise “Research and Practical Center of the National Academy of Sciences of Belarus for Animal Breeding” (Zhodino, Republic of Belarus). According to this aim, three experiments were carried out, depending on the breeding season of animals and hormonal preparations used for synchronization:

- Synchronization-and-stimulation of estrus in recipient goats was carried out with an analogue of prostaglandin F₂α fertadine at a dose of 150 μg twice with an interval of 12 days. The treatment was carried out at different periods of estrus activity of animals throughout the year.
- Use of intravaginal sponges containing 40 mg of progesterone for 6, 9 and 12 days in combination with PMSG and fertadine at doses of 500 IU and 150 μg, which were injected intramuscularly 48 and 24 hours before removal of the implant, in the estrus stimulation schemes in recipient goats, respectively.
- Analysis of the developed schemes based on the use of prostaglandin F₂α analogue fertadine and progestogenic medicines in terms of acceptability rate of goat biomaterial. Goats of the 1st experimental group were treated with fertadine twice with an interval of 12 days. The animals
of the 2nd experimental group were injected intravaginally with pessaries. The control group consisted of dams that came to spontaneous estrus from September to November.

Determination of estrus signs of in goats was carried out twice (in the morning and in the evening) using test goats.

Transplantation of biomaterial of excellent and good quality at the preimplantation stages of development (morula-blastocyst) obtained from clinically healthy mature transgenic donor goats (carrying a DNA construct in the genome (rhLf5 35013 bp length, containing a copy of the genomic sequence of human lactoferrin under the control of the beta-casein promoter of goats, repeat section from two insulators of chicken beta-globin gene, untranslated sections), with the content of recombinant human lactoferrin in milk 2-3 g/l was taken to the genital tract of female recipients (clinically healthy nontransgenic and transgenic goats of breeding age with body weight of 35-40 kg) into the apex of the uterine horn on the side of ovary with a well-defined corpus luteum (ipsilateral).

Based on the results of embryo acceptability and goatlings yield, efficient scheme of synchronization-and-stimulation of estrus in recipient goats was determined.

3. Research results
To date, various hormonal schemes have been developed for influencing the reproductive cycle of small ruminants. Choice of the scheme and method of regulation depends on the breeding season and time of the year. For instance, prostaglandins are used during the breeding season when females have functional corpus luteum in the ovaries. Hormonal preparations in estrus stimulation schemes in goats and sheep are used mainly out of the breeding season.

In this regard, in the first experiment, a comparative assessment of acceptability rate of biomaterial of goats, depending on the estrus activity of animals during the breeding season, was carried out. The research results are shown in table 1.

Table 1. Acceptability of biomaterial of goats depending on the breeding season of animals.

| Parameter                              | Breeding season |
|----------------------------------------|-----------------|
|                                        | Sep-Nov | Dec-Mar |
| Goats treated, animals/%               | 20/100.0  | 20/100.0 |
| Showed estrus in 72 hours, animals/%   | 18/90.0   | 14/70.0  |
| Number of transplants/embryos          | 18/36     | 14/28    |
| Acceptability on the 40th day, animals/%| 11/61.1  | 6/42.9   |
| Acceptability as of goating, animals/% | 10/55.6  | 6/42.9   |
| Obtained goatlings, animals             | 14        | 6        |
| Fertility, %                           | 140.0     | 100.0    |

It has been determined that the breeding season during the calendar year affected the rate of embryo acceptability. So, in the group of goats treated in the period from September to November (active breeding period), 90.0% of animals showed estrus (18 out of 20), while in the group of December-March (inactive period), this indicator made 70.0% (14 out of 20). Acceptability rate on the 40th day of pregnancy diagnosis after transplantation of two embryos to each recipient made 61.1% in the first group and 42.9% in the second one. As of the goating moment, the acceptability rate in the estrous period made 55.6% (10 out of 18), and in the anestral period – 42.9% (6 out of 14) and fertility – 140.0%, which is 40 p.p. higher in comparison with the second group of goats (100.0%). Intravaginal sponges impregnated with synthetic analogues of progesterone are widely used as hormonal agents for synchronizing estrus in small ruminants during and out of the breeding season. In this regard, at the second stage of the research, a comparative characteristic of indicators of embryo acceptability and yield of goatlings was carried out, depending on the time of use of progestogen inserts (6, 9 and 12 days). The research results are shown in table 2.
Table 2. Acceptability of biomaterial of recipient goats depending on the duration of use of intravaginal sponges when stimulating estrus in animals.

| Parameter                                      | Implant exposure period, days |
|------------------------------------------------|-------------------------------|
|                                                | 12               | 9            | 6            |
| Goats treated, animals/%                       | 15/100.0         | 15/100.0     | 15/100.0     |
| Showed estrus in 30 hours, animals/%           | 15/100.0         | 12/80.0      | 15/100.0     |
| incl. from those in estrus:                    |                  |              |
| in 12 hours                                    | 5/33.3           | 3/25.0       | 0/0          |
| in 20 hours                                    | 8/53.3           | 0/0.0        | 3/20.0       |
| in 30 hours                                    | 2/13.3           | 9/75.0       | 12/80.0      |
| Number of transplants/embryos                  | 15/30            | 12/24        | 15/30        |
| Acceptability on the 40th day, animals/%       | 9/60.0           | 7/58.3       | 8/53.3       |
| Acceptability as of goating, animals/%         | 8/53.3           | 6/50.0       | 7/46.7       |
| Obtained goatlings, animals                    | 11               | 8            | 11           |
| Fertility, %                                   | 137.5            | 133.3        | 157.1        |

It has been determined that the period of exposure to intravaginal inserts influenced the main studied parameters: the number of animals that showed signs of estrus, the time of the onset of estrus, acceptability in different periods and the yield of goatlings. Thus, the 12-day synchronization scheme for estrus in recipient dams made it possible to obtain 100.0% of animals that came into estrus within 30 hours from the moment the implant was removed, including 86.6% in 20 hours. While in the group of animals, where the period of use of the implant was 6 and 9 days, 20.0-25.0% of goats showed estrus within 20 hours. The main number of animals came into estrus only after 30 hours and amounted to 75.0-80.0%. This circumstance shall be taken into account when forming groups of animals when carrying out planned work on the extraction and transplantation of embryos in the synchronization schemes for the estrus of donors and recipients. Acceptability of embryos on the 40th day of pregnancy diagnostics made 60.0% (9 out of 15) in the group with the duration of device installation within 12 days; 9 days - 58.3% (7 out of 12) and 6 days - 53.3% (8 out of 15). In all groups, as of goating moment, a delay in fetal development was recorded in prenatal ontogenesis, in the first group 8 dams out of 9 possible gave birth, in the second – 6 out of 7 and in the third – 7 out of 8. In the group with the 12 days duration of exposure to intravaginal sponges, a total of 11 goatlings from 8 dams were received, in the group of 9 days – 8 from 6 that gave birth and in the group of 6 days – 11 from 7, while the fertility rate made 137.5; 133.3 and 157.1%, respectively. Comparative characteristics of acceptability parameters of the biomaterial of goats in recipient animals, depending on the method of using hormonal preparations and their complexes, are presented in table 3.

Table 3. Acceptability of goat embryos depending on the method of synchronization of estrus in recipient animals.

| Parameter                        | Experiment (Group I) | Experiment (Group II) | Control (spontaneous estrus) |
|----------------------------------|----------------------|-----------------------|------------------------------|
| Goats used, total, animals       | 40                   | 45                    | 30                           |
| Goats treated, animals/%         | 40/100.0             | 45/100.0              | 30/100.0                     |
| Showed estrus in 72 hours, animals/% | 32/80.0            | 42/93.3               | 8/26.7                       |
| Number of transplants/embryos    | 32/64                | 42/84                 | 8/26.7                       |
| Acceptability on the 40th day, animals/% | 17/53.1             | 24/57.1               | 19/63.3                      |
| Acceptability as of goating, animals/% | 16/50.0            | 21/50.0               | 17/56.7                      |
| Obtained goatlings, animals      | 20                   | 30                    | 22                           |
| Fertility, %                     | 125.0                | 142.9                 | 129.4                        |
When analyzing the data in table 3, we can conclude that the use of fertadine allowed to bring up to 80.0% of animals into estrus, and use of intravaginal sponges up to 93.3% of animals, while in 72 hours only 26.7% of recipient animals came to natural estrus during the estrous period. The acceptability rate on the 40th day after embryo transplantation was higher in the control group and amounted to 63.3%, which is by 6.2 and 10.2 percentage points higher than in the second and first groups, respectively. The acceptability as of the goating moment in all experimental groups was the same and amounted to 50.0%, in the control group the same indicator was at the level of 56.7%. Multiple goatings were recorded in all groups. In the first group, 20 goatlings were obtained from 16 goatings, in the second, from 21 - 30 goatlings, and in the control group, 22 goatlings were obtained from 17 animals. Fertility rate was higher in the group where synchronization of estrus was carried out using progestogen implants, and amounted to 142.9%, while in the first group this indicator was at the level of 125.0%, and in the control group – 129.4%. Thus, the use of prostaglandin F₂α preparations during the estrous period of reproduction (September-March) and progestogenic implants, irrespective of the breeding season during the year, in the synchronization schemes for estrus in recipient animals, makes it possible to achieve acceptability rate of 50.0% and offspring yield of 135.1 %.

4. Conclusion
Use of prostaglandin F₂α – fertadine – in the synchronization-and-stimulation schemes of estrus twice with an interval of 12 days at a total dose of 300 μg of the active substance contributed to obtaining of embryo acceptability at the level of 55.6% with yield of 140.0% of goatings taken into account the season of reproduction. The combined use of intravaginal progestogen pessaries impregnated with 40 mg of progesterone with fertadine at a dose of 150 μg and PMSA at a dose of 500 IU made it possible to induce estrus in 100% of animals within 30 hours and to obtain embryo acceptability rate of 50.0% with a fertility of 142.9%, regardless of breeding season throughout the year.

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