eISSN: 09748369

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Biology and Medicine

Research Article

Volume 6, Issue 1, Article ID: BM-008-14, 2014

Indexed by Scopus (Elsevier)

Co-Publisher: OMICS Group, www.omicsonline.org

www.biolmedonline.com

Influence of sexual season on implantation of microinjected embryos and birth rate of new born-transplant in experiments on creating transgenic goats carrying human lactoferrin gene

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Received: 25th Jul 2014; Accepted: 3rd Sep 2014; Published: 12th Sep 2014

Abstract

The article presents the results of the study on the viability of microinjected embryos in goats, as well as determining the level of engraftment of transplanted embryos, depending on the season. Also, a new scheme of getting even-aged oocytes at the stage of two pronuclei was proposed. Polyovulation in donor goats amounted to 16.4 of corpus luteum per head. In addition, 11.8 oocytes (embryos)/head were recovered, 87.5% of them being presented by the fertilized oocytes at the stage of two pronuclei. During the transplantation of embryos after microinjection of gene construction, their acceptability was low and amounted to an average of 16.3% (lim 8.3-27%). In sexual season, the acceptability was 3.25 times higher than in nonsexual season. Fetal lysis of already implanted embryos was averaged to 13.7%.

Keywords: Goat; oocyte; pronuclear; embryo; transplantation; transgenesis; gene construction; microinjection; oestrus; sexual season; polyovulation; implantation; delivery.

Introduction

The essence of transgenesis is that the gene construct of any animal species is able to function, synthesizing encoded proteins after its genome integration with other animal species (genus or even phylum) [1-3]. The most used method of transgenesis in creating transgenic animals is the method of microinjection. The essence of microinjection is the introduction of a solution of gene construction directly into the pronucleus of the zygote [4,5]. Embryo transplantation is a complicated biotechnological method with a sufficiently low efficiency compared with other reproduction methods [6,7]. Given that microinjection of the foreign DNA is a critical intervention in vital functions of gamete, we can predict the reduction of its vitality, and hence the ability for implantation. The objectives of the present study were to clarify the viability of microinjected embryos in goats, as well as to determine the effectiveness of acceptability of transplanted embryos (development of pregnancy culminating in the delivery) depending on the season.

Methods

Experiments were performed in the sexual season (November), at the end of the sexual season (December), and in the ancestral period (March-April).

Preparation of donors and receiving embryos

Saanen goats aged 2.5-3.5 yrs were used as donors. Polyovulation in donors was induced by the following scheme:

1. Synchronization of the estrous cycle (ear implants "Crectar", Intravet, dose 1.5 mg/head, the dosage was estimated by an active compound norgestimate, implant application for 12 days).
2. Interruption of the luteal phase of estrous cycle (drug "Folligon", Intravet in the dose of 500 IE/head, single subcutaneous injection).
3. Stimulation of oogenesis (drug "Ovagen", Intravet, starting with 60 h from implant extraction, a total dose of 5.6 mg/head. Fractional subcutaneous injections of 0.8 mg/goal 12 h apart. Beginning of injections in 60 h before removing the implant, the dosage was estimated by an active compound NIADDK-oFSH-17).
4. Providing synchronous ovulation (drug "Chorulon", Intervet, 500 IE/head, single intramuscular injection in 4 h after beginning of oestrus).

The period of the onset and duration of oestrus was determined using the reflexology method using tester goats. Selection of goats for oestrus was started after 12 h after implants removal and performed every 2 h apart until the end of oestrus. Intracervical insemination of goats was performed by 0.2 ml fresh sperm. Preparation of fertilized oocytes in donor goats was performed by laparotomy. Embryos were extracted through the funnel of the oviduct. For this purpose, the washing liquid under pressure of the piston was injected into the cavity of the uterine horn in such a way that it is flowed out of the funnel of the oviduct in a special container after passing through the tip of the horn and oviduct. KSOM was used as the washing liquid. Terms of laparotomy varied and depended on the duration of oestrus in donors. When the duration of oestrus was 15-22 h, an operation was carried out in 40-44 h after the start of the oestrus; when the duration of oestrus was 23-36 h, an operation was carried out in 48-54 h after the start of the oestrus.

Preparing of the recipients

Indigenous goats aged 1.5-4 yrs were used as recipients. The drug "Estrofan", Biovet was used in the dose of 125 mg/head in order to prepare the recipient. The dosage was estimated by an active compound cloprostenol, and two fold subcutaneous injections with an interval of 12 days were made.

Microinjections

Gametes were sought out in the collected lavage fluid followed by their initial assessment. Oocytes were centrifuged (10000 rev/min, 15 min) in order to visualize the pronuclei. If necessary, the cells were allowed to culture in an incubator at 5% CO_2 and 39°C in KSOM medium under the paraffin oil layer. The gene construction was introduced into one of the pronuclei by microinjection method. The following gene construction was used: hLf5 by the sites XhoI-NotI into the vector pBC1 resulted in cloned genomic sequence of lactoferrin 35013 bp in length, starting with ATG codon. After that the microinjection oocytes were cultivated *in vitro* until obtaining 2-4 blastomeric embryos.

Embryo transplantation

Embryo transplantation was performed by laparotomy. 2-3 Embryos were transplanted to each recipient in the upper third of the oviduct corresponding to the ovulating ovary. To do this, a cannula with an embryo was introduced through the oviduct funnel into the oviduct to a depth of 4-5 cm followed by an embryo injection. Embryo implantation in the recipient goats was determined in 20 days after transplantation by the study of progesterone concentrations in the blood of animals. Further development of the fetus was monitored by an ultrasound examination. Finally, acceptability of microinjected cells was determined by the results of a birth rate.

Determination of transgenic offspring

The presence/absence of the hLf gene in newborn goats was determined by polymerase chain reaction.

Main Part of the Study

During the preparation of donors an oestrus was revealed in 15 (79%) of 19 treated goats. An oestrus was determined in the presence of the full manifestation of all its phenomena (positive reaction to the male animal, estruation, immobility reflex). At the same time, an oestrus began on average in 16.6 h after removal of the implant and lasted for 23 h. In all animals in the estruation period, there was an effect of polyovulation. In the average polyovulation was 16.4 lutea per donor (Table 1).

Table 1: Results of polyovulation induction in donor goats.

During the experiment	Number of donors	Came into oestrus, head/%	Responded polyovulation from animals in oestrus, head/%	From time of implant extracting to the oestrus, hours (M ± m)	Duration of the oestrus, hours (M ± m)	Reaction of polyovulation, number of corpora lutea (M ± m)
November	3	3/100	3/100	16.7 ± 2.31	19.77 ± 4.73	14.7 ± 3.22
December	2	7/77.8	7/100	14.5 ± 1.98	27.8 ± 6.59	16.0 ± 5.76
March-April	7	5/71.4	5/100	18.6 ± 6.62	21.2 ± 7.80	18.4 ± 7.10
Total	1	15/78.95	15/100	16.6 ± 1.18	23.0 ± 1.94	16.4 ± 1.62

A total number of 177 zygotes were obtained during the experiment. On average, 11.8 zygotes were obtained from one donor. Among them, on average 87.5% were suitable for microinjection (fertilized oocytes at the stage of two pronuclei); 12.5% were unfertilized and degenerating oocytes as well as embryos at the stage of two blastomers. Percentage of oocytes suitable for microinjection was higher in the experiments conducted during and at the end of the sexual season: 90.3% and 94%, while the percentage of unfertilized and degenerating oocytes were 3.2% and 6%, respectively. In asexual season oocytes suitable for microinjection amounted to 73.5% of all the extracted ones, 26.5% of them being unfertilized and degenerating oocytes (Table 2).

An acceptability of microinjected embryos is presented in Table 3. The study of progesterone concentrations in blood serum of recipient goats revealed that an average of 40% of goats became pregnant in 20 days after transplantation: 44.4% in November experiment, 40.1% in December experiment, and 33.3% in the experiment conducted in spring. However, ultrasound examination in 2 months after transplantation revealed that only 25% of goats become pregnant on average. That is, violation of embryonic development and embryolysis occurred in 37.5% of the animals with already implanted embryos in the early prenatal period.

Another case of developmental disorders in the late prenatal period that resulted in abortion was also noted. Three fetus were extracted: one was normally developed, consistent with its gestational age, two aborted at different stages of development, with deformed mandibles and signs of maceration. Fecundity of recipient goats ranged from 22.2% to 55.6%, respectively.

According to the results of PCR analysis, there were no transgenic goats among transplants. All newborn goats were normally developed, had emphasized sucking reflex.

Table 2: Results of oocytes/embryos extraction in donor goats.

Period of the experiment	Number of donors	Extracted oocytes/ embryos in one donor (M ± m)	Of these		
			Oocytes (M ± m/%)		Embryos on the stage of 2 blastomeres (M ± m/%)
			Suitable for microinjection	Unfertilized and degenerating	
November	3	10.3 ± 7.02	9.3 ± 8.62/90.3	0.3 ± 0.33/3.2	0.7 ± 0.67/6.5
December	7	13.8 ± 6.71	13.0 ± 7.56/94.0	0.7 ± 0.57/6.0	–/0
March-April	5	9.8 ± 5.36	7.2 ± 2.68/73.5	2.6 ± 1.92/26.5	–/0
Total	1	11.8 ± 1.64	10.3 ± 1.75/87.5	1.4 ± 0.70/11.3	0.1 ± 0.13/1.1

Table 3: Level of implantation of microinjected embryos.

Parameters	Period of the experiment		
	November	December	March-April
Operated recipients	9	22	9
Transplanted cells	18	51	24
Became pregnant, head/%	4/44.4	9/40.1	3/33.3
Fetal lysis, head/%	1/11.1	4/18.2	1/11.1
Developmental disorder in the late prenatal ontogenesis, head/%	1/11.1	–	–
Hormonal disorders (hydrometer), head/%	1/5.5	–	–
Pregnancy, culminated with delivery, head/%	2/22.2	5/22.7	2/22.7
Born goals, head	5	7	2
Of these transgenic goals, head/%	–	–	–
Fecundity of recipient goats, %	55.6	31.8	22.2
Acceptability of microinjected cells, %	27	13.7	8.3

Growth and development of goatling occurred in accordance with standards.

Conclusion

The obtained results allow to state that the developed scheme of induction of polyovulation gave good results with a high degree of synchronization of ovulation, as confirmed by a high percentage of cells of the same age. An effective polyovulation resulted in the average of 16.4 corpora lutea/head. The number of extracted oocytes reached 11.8 zygotes/head, 87.5% of them being in the stage of two pronuclei that greatly exceeds the known results [4,5,8-10].

Acceptability of transplanted embryos after microinjection of gene construction into them averaged to 16.3%. In the sexual season, the acceptability was 3.25 times higher than that in nonsexual season (27% and 8.3%, respectively), while the fecundity of goats was higher by 2.5 times.

Fetal lysis of the already implanted embryos ranged from 11.1% to 18.2%. The possibility of developmental abnormalities and fetal death in early (mostly), and in late prenatal period was estimated.

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Citation: Aksenova PV, Ermakov AM, Kartashov SN, Mironova LP, Klimenko AI, *et al.* (2014) Influence of sexual season on implantation of microinjected embryos and birth rate of new born-transplant in experiments on creating transgenic goats carrying human lactoferrin gene. Biol Med 6(1): BM-008-14.