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ДОКЛАДЫ

НАЦИОНАЛЬНОЙ АКАДЕМИИ НАУК РЕСПУБЛИКИ КАЗАХСТАН

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CONSERVATION OF THE GENE POOL OF ENDANGERED ARGALI SPECIES

Abstract. Argali living on the territory of the Republic of Kazakhstan are listed in the Red Book and need special protection of the state. The number of these animals is falling every year. Urgent measures are being taken to preserve the existing species of argali in all their genetic diversity. One of the promising methods of preserving the gene pool of endangered species of wild animals is the method of deep freezing of sperm and embryo transplantation.

The argali or mountain sheep species includes several subspecies that are quite well studied and differ in their external characteristics:

- altai ram or Ovis ammon ammon:
- anatolian mouflon or Ovis ammon anatolica;
- bukhara ram or Ovis ammon bocharensis;
- kazakh argali or Ovis ammon collium;
- gansu argali or Ovis ammon dalailamae;
- tibetan mountain sheep or Ovis ammon hodgsonii;
- north China mountain sheep or Ovis ammon jubata;
- tianshan mountain sheep or Ovis ammon karelini;
- argali Kozlova or Ovis ammon kozlovi;
- karatau mountain sheep or Ovis ammon nigrimontana;
- cyprus ram or Ovis ammon ophion;
- mountain ram marco polo or Ovis ammon polii;
- kyzylkum mountain sheep or Ovis ammon severtzovi;
- urmian mouflon or Ovis ammon urmiana.

Conservation of biological diversity around the world is now deservedly given great attention. The problem of accelerated reproduction of genetic resources has become particularly relevant at the present time, when the Red Book is becoming larger every year, and the world around us is becoming poorer. Every year, the planet loses many species of animals and plants. Therefore, the search for conservation opportunities for endangered populations of wild animals is not only of scientific, but also of great practical interest.

The theoretical basis for the conservation of rare and endangered species of wild animals in Kazakhstan has not yet been developed and has not yet been sufficiently studied. The use of biotechnological techniques makes it possible to preserve the genetic basis of valuable and endangered animal species.

Key words: induction, poliovulation, synchronization, recipient sheep, embryo, injection, insemination, transplantation, argali.

Introduction Argali in Kazakhstan or mountain sheep. The main habitats of argali in Kazakhstan are the mountains of Karatau, Tien Shan, Dzungarian Alatau, Tarbagatai, Saur, Kalba and Southern Altai Mountains, Chu-Ili Mountains and the Kazakh Highlands [1, p. 1].

Appearance of argali: The body length of an adult argali is 120-200 cm, with a height at the withers of 90-120 cm and a weight of 65-180 kg. Depending on the subspecies, not only the size, but also the color of the body vary, but by far the largest is the Pamir argali, or mountain ram Marco Polo, which got its name in honor of the famous traveler who gave the first description of this mammalian artiodactyl animal.

Males and females of this subspecies are characterized by the presence of very long horns. The male mountain ram has larger, more impressive horns, which often weigh almost 13% of the total body weight

of the animal. Horns, up to 180-190 cm long, spirally twisted, with the ends turned outwards and upwards. The coloration of the body of a semi-horned artiodactyl mammal can vary significantly, which is due to the characteristics of the subspecies. Most often, the color is represented by a very wide range from light sandy shades to dark grayish-brown.

The argali or mountain sheep species includes several subspecies that are quite well studied and differ in their external characteristics:

- altai ram or Ovis ammon ammon:
- anatolian mouflon or Ovis ammon anatolica:
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- cyprus ram or Ovis ammon ophion;
- mountain ram marco polo or Ovis ammon polii;
- kyzylkum mountain sheep or Ovis ammon severtzovi;
- urmian mouflon or Ovis ammon urmiana [2, p. 1].

Of particular interest is the subspecies of argali-Altai or Tien Shan mountain sheep. This artiodactyl mammal, belonging to the family of bighorn sheep, has the most powerful and very heavy horns. The average weight that the horns of an adult male have, often reaches 33-35 kg. The height of a mature male at the withers can vary between 70-125 cm, with a body length of up to two meters and a weight of 70-180 kg. The length of the tail is 13-14 cm. All representatives of the subspecies O. ammop ammop are characterized by the presence of a rather squat body, thin, but very strong limbs. The end of the nasal mirror of the animal has a lighter color than its head and back. The population of the Altai mountain sheep can be represented by two main groups: females with young individuals and sexually mature males.

No less interesting is the mountain Kyzylkum sheep or the argali of Severtsov. This endemic of the territory of Kazakhstan is currently under threat of complete extinction, and the number of this subspecies does not exceed one hundred individuals. Ovis ammop severtzovi is listed in the Red Book, operating on the territory of Kazakhstan.

Habitat: They live in the south of Siberia, in Central and Central Asia (Sayans, Tibet, Himalayas, Pamirs).

Lifestyle: They live in small groups, gathering in herds for the winter. Females and their young are kept separate. They actively graze in the morning and in the evening, rest day and night, forming rookeries. They hide among rocks or mountains, thus preventing the penetration of predators. In winter, they have to feed all day, digging up grass under the snow – not an easy task, and a lot of energy is spent.

Food: Wild mountain sheep argali belong to the category of herbivores, which is why the main diet of artiodactyls is represented by a diverse, herbaceous vegetation that is characteristic of the area and area in which the subspecies exists.

Breeding and mating season: Argali breed from late autumn to early winter. The rut period falls on the period from October to January. The mating season passes very quietly, they do not arrange battles for the female, do not show each other their luxurious horns. By the time of the mating season, there are not a large number of males and females in the group. After 5 months, around June, small defenseless lambs are born. The female usually gives birth to one lamb, two or three-this is already rare.

On the 5th day, the lamb is already on its feet and follows its mother everywhere. It feeds exclusively on its mother's milk. Well, when he turns a month old, he begins to eat grass as well. Completely independent of the lamb will be the next spring. He will be considered an adult when he is three years old, and the weight of an adult male will gain only by the age of 7. At the age of 6, he grows teeth and horns.

Life span: The average life span of a mountain ram or argali can vary greatly depending on many external factors, including the area of distribution. But, as a rule, in natural, natural conditions, the artiodactyl polorogoe mammal animal is able to live no more than ten or twelve years.

Problems with preserving the view: Wild mountain sheep Argali and all its subspecies are very small, some are threatened with complete extinction, so they are listed in the Red Book of many countries, including Russia, Kazakhstan, Mongolia, and China. It is forbidden not only to hunt animals, but also to sell skins, horns and other parts of the carcass. Despite all the protective measures, the number of animals is constantly decreasing. The Dagestani population and the plight of Argali from the Kyzylkum desert have almost disappeared.

Argali take root well in zoos and give healthy offspring. This gives hope that over time, it will be possible to populate with new individuals areas in which the herds have long disappeared.

Conservation of biological diversity around the world is now deservedly given great attention. The problem of accelerated reproduction of genetic resources has become particularly relevant at the present time, when the Red Book is getting bigger every year, and the world around us is getting poorer. Every year, the planet loses many species of animals and plants. Therefore, the search for conservation opportunities for endangered populations of wild animals is not only of scientific, but also of great practical interest [3, p. 99-101].

The theoretical basis for the conservation of rare and endangered species of wild animals in Kazakhstan has not yet been developed and has not yet been sufficiently studied. The use of biotechnological techniques makes it possible to preserve the genetic basis of valuable and endangered animal species.

Of great scientific and practical interest is the solution of issues of conservation and accelerated reproduction of wild argali in Kazakhstan, where they are on the verge of extinction through the use of modern biotechnological methods [4, p. 35-41].

Argali living on the territory of the Republic of Kazakhstan are listed in the Red Book and need special protection of the state. The number of these animals is falling every year. Urgent measures are being taken to preserve the existing species of argali in all their genetic diversity.

Materials and methods of research. Polyovulation induction and synchronization of sexual cycles of the females rams with the ewes recipient, surgical washout and embryo transfer.

The experimental work was carried out in the Almaty zoo, where 8 heads of female argali belonging to the subspecies of argali were kept Ovis ammon carelini.

Female argali were given a small dose of a tranquilizer intramuscularly to relieve stress before all the procedures performed: "Promazin" (Promasin, USA).

Argali kept in the zoo are as wild as in the mountains, and therefore it is very difficult to determine the duration of the estrous cycle in female argali. The rut periods of wild argali vary in different parts of the range and range from mid-October to mid-January, and vary greatly by individual years. To synchronize the sexual cycles of donors and recipients, we used intervaginal sponges impregnated with progesterone.

The donor (female argali) and the recipient (female sheep) were synchronized according to the estrous cycle by an artificial method using vaginal sponges impregnated with 60 mg of methoxyprogesterone acetate (MAP). On the 12th day of the sexual cycle, 48 hours before the removal of the sponges, two donors received a single intramuscular injection of 1200 IU of HCG.

24 hours after the removal of the sponges, before insemination, 1000 IU of chorionic gonadotropin was administered intravenously to the female argali for synchronous ovulation of the follicles (table 1). After which the male argali was admitted to the females.

Day	Medication	Drug doses	
		The donor	Recipient
zeroth	Introduction of intravaginal sponges	60 mg	60 mg
eleventh	HCG Injection	1200 International unit	-
fourteenth	Estrus and human chorionic gonadotropin. Insemination.	1000 International unit	
nineteenth	Extraction of embryos		

Table 1 - Scheme of synchronization and hormonal treatment of female argali donors

Surgical operation to wash out embryos from female donors of argali and their transplantation to sheep recipients was performed surgically by laparotomy along the white line of the abdomen using local anesthesia, according to the generally accepted method used for washing out embryos in domestic sheep. Before the operation, donors and recipients were kept on a starvation diet for 24 hours. The technique of the surgical operation was as follows: the animal was fixed on the operating table in a dorsal position. The operating field was cut with scissors first and then a razor to shave the area to remove the hair and the top are covered by the operating bed sheets incision in the operating area. The shaved skin was decontaminated and lubricated with a 5% solution of iodine. Up to 10 ml of 0.5-2% novocaine solution was injected along the incision line and an incision of 5-6 cm long was made at a distance of 2-3 cm from the anterior edge of the breast. The abdominal wall was opened along the white line of the abdomen. With the middle and index fingers of the hand, the horns of the uterus and the ovaries were found and extracted. In both ovaries was determined by the number of yellow bodies and Novoulyanovsk follicles.



Figure 1 - Extraction of the uterine horns from the abdominal cavity of the donor



Figure 2 - Leaching of multiple embryos in a female argali

The embryos were washed with the medium by washing through a 7-8 cm long plastic tube fixed in the febrile end of the oviduct. The washing liquid was collected in special Petri dishes, in which the number of embryos was counted under a microscope. The resulting embryos were evaluated by morphological characteristics immediately after detection under a microscope. This takes into account the

main morphological features of the usefulness of embryos: the integrity and uniformity of development of blastomeres, transparency perivitelline space, the integrity of the area pellucida of sootvetstviya stages of development age of the embryo.

Full-fledged embryos were considered to have a regular spherical shape, homogeneous cytoplasm, with a complete transparent shell, and the same size of blastomeres.

The defective embryos were shrunken, with uneven and deformed blastomeres and a lag in crushing.

Embryo transplantation to recipients was also performed surgically in the ipsilateral side of the uterine horn. Before the operation, the recipient ewes were kept on a starvation diet for 24 hours. The technique of the work was as follows: the animal was fixed on the operating table in a dorsal position. The operating field was cut with scissors first and then a razor to shave the area to remove the hair and the top are covered by the operating bed sheets incision in the operating area. The shaved skin was decontaminated and lubricated with a 5% solution of iodine. Up to 10 ml of 0.5-2% novocaine solution was injected along the incision line and a 5-6 cm incision was made at a distance of 2-3 cm from the anterior edge of the breast. The abdominal wall was opened along the white line of the abdomen. With the middle and index fingers of the hand, the horns of the uterus and the ovaries were found and extracted. Before embryo transfer, the ipsilateral side of the uterine horn was determined, i.e., the side of the horn in which ovulation occurred and the yellow body was formed. For embryo transplantation, a pipette with a glass capillary was used, with which a solution with embryos was injected. The embryos were recruited into the capillary in the following way: medium-air-medium with embryos-air-medium and introduced into the oviduct through a funnel. In the upper third of the uterine horn by piercing with special needles. With a blunt end and the introduction of a capillary with embryos to a depth of 1.5-2 cm horns. After embryo transplantation, the recipient's oviduct and uterus were irrigated with a solution of penicillin and streptomycin and catgut sutured first the parietal peritoneum, transverse and superficial fascia, and then the subcutaneous tissue. The wound area was treated with a 5% iodine solution.

Research results and discussion. As a result of hormonal treatment, it was found that when treating female argali with these hormones, the best super ovulation response was shown by animals treated with serum hormone of foaled wild boars (HSHC) in contrast to follicle stimulating hormone (FSH) (Table 2).

Thus, the positive reaction of the superovulation of animals from the number of treated serum hormone of foaled wild boars was 100 % or 2 female argali and with the introduction of follicle stimulating hormone 50% of animals or 1 female argali. The average number of ovulations per donor was higher in the treatment of female argali with serum hormone of foaled wild boars (6.0 ± 2.3) , compared to the administration of FSH (4.5 ± 1.1) .

Thus, as a result of the use of these schemes of hormonal treatments with serum hormone of foaled wild boars and FSH for the induction of superovulation of 75% of the treated animals, 20 ovulations per seven animals were obtained, which on average amounted to 5.25±0.62 ovulations per donor.

The resulting embryos were detected under the stereoscopic microscope MBS-9. The assessment was carried out by morphological features, using an inverted microscope of the company «Leica» at a hundredfold magnification.

		Gonadotropins			
Indicator	Serum hormone of foaled wild boars		Follicle stimulating hormone		
	n	%	n	%	
Processed animals, heads	2	100	2	100	
Reacted by superovulation, n %	2	100	1	50	
The number of ovulation, just	12	-	8	-	
Number of ovulations per donor	6,0±2,3	-	4,5±1,1	-	
Novoulyanovsk follicles, n	3	-	10	-	
Embryos extracted, total %	8	66,6	6	75	
Unfertilized eggs, %	2	29	1	16,6	
Degenerated eggs and embryos	3	37,5	1	16,6	

Table 2 - Results of induction of superovulation of female argali depending on the type of gonadotropin.



Figure 3 - Female argali selected for the induction of poliovulation

Of the 14 washed eggs and embryos, 3 or 21.4% were unfertilized, 4 (28.5%) embryos were rejected from the number of fertilized and developing to a certain stage of crushing according to morphological characteristics. Taking into account 3 unfertilized eggs, 7 embryos and eggs were unsuitable, which is 50 %.

Thus, 7 embryos or 50% are considered suitable for transplantation.

Embryo transplants to recipient animals were also performed surgically by laparotomy. The embryos were transplanted into the oviduct or into the horns of the uterus in the ipsilateral side of the ovary with a functioning yellow body.

Early and late moruls (96-120 hours) were transplanted with a Hamilton syringe micropipette into the upper part of one of the uterine horns after piercing its wall.

As a result, after 5 months, the recipient sheep was successfully treated with an argali graft.

Conclusions. Thus, it was found that the sheep can be used as a surrogate mother for carrying argali pups and, accordingly, the use of the method of superovulation and embryo transplantation allows for accelerated reproduction of the population of endangered subspecies of argali.

А. Р. Сансызбай, М. Умитжанов, Н. Ж. Бакиров, М. Б. Рысбаев

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ЖОЙЫЛЫП БАРА ЖАТҚАН АРХАР ТҮРЛЕРІНІҢ ГЕНДІК ҚОРЫН САҚТАУ

Аннотация. Қазақстан Республикасының аумағында кездесетін арқарлар Қызыл кітапқа енгізілген және мемлекеттің ерекше қорғауын қажет етеді. Бұл жануарлардың саны жыл сайын азайып келеді. Қазіргі уақытта архардың барлық генетикалық әртүрлілігінде бар түрлерін сақтау үшін шұғыл шаралар қабылдануда. Жойылып бара жатқан жабайы жануарлардың гендік қорын сақтаудың перспективті әдістерінің бірі — шәуітті (сперматозоидтарды) терең мұздату және эмбриондарды трансплантациялау әдісі.

Арқар немесе тау қойларының түрлеріне бірнеше жақсы зерттелген және сыртқы сипаттамалары бойынша ерекшеленетін түрлер кіреді:

- Алтай қошқарлары немесе Ovis ammon ammon;
- Анатолий мофлоны немесе Ovis ammon anatolica;
- Бұхар қойы немесе Ovis ammon bocharensis;
- Қазақстандық аргалы немесе Ovis ammon collium;
- Ганьсу аргалы немесе Ovis ammon dalailamae;
- Тибет тау қойы немесе Ovis ammon hodgsonii;
- Солтүстік Қытай тау қойы немесе Ovis ammon jubata;
- Тяньшань тау арқары немесе Ovis ammon karelini;
- Козлова арғалы немесе Ovis ammon kozlovi;

- Қаратау тау қойы немесе Ovis ammon nigrimontana;
- Кипрлік қошқар немесе Ovis ammon ophion;
- Марко Поло тау қошқары немесе Ovis ammon polii;
- Қызылқұм тау қойы немесе Ovis ammon severtzovi;
- Урма мофлоны немесе Ovis ammon urmiana.

Қазір бүкіл әлемде биологиялық әртүрлілікті сақтауға көп көңіл бөлінеді. Қазіргі уақытта Қызыл кітап жыл сайын көлемді болып, айналамыздағы әлем кедей генетикалық ресурстардың жедел көбеюі мәселесі ерекше өзекті болды. Жыл сайын планета бойынша жануарлар мен өсімдіктердің көптеген түрлері жоғалып кетуде. Сондықтан жабайы жануарлар популяциясының жойылу қаупінде тұрған сақтау мүмкіндіктерін зерттеу тек ғылыми ғана емес, сонымен бірге үлкен практикалық қызығушылыққа ие.

Қазақстандағы жабайы жануарлардың сирек кездесетін және жойылып бара жатқан түрлерін сақтаудың теориялық негіздері әлі күнге дейін әзірленбеген және әлі жеткілікті зерттелмеген. Биотехнологиялық әдістерді қолдану жануарлардың құнды және жойылып бара жатқан түрлерінің генетикалық негізін сақтауға мүмкіндік береді.

Түйін сөздер: индукция, полиовуляция, синхрондау, аналық қой, эмбрион, инъекция, ұрықтандыру, трансплантация, арқарлар.

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СОХРАНЕНИЕ ГЕНОФОНДА ИСЧЕЗАЮЩИХ ВИДОВ АРХАРОВ

Аннотация. Архары, обитающие на территории Республики Казахстан, занесены в Красную книгу и нуждаются в особой защите государства. Численность этих животных с каждым годом падает. В настоящее время принимаются срочные меры по сохранению существующих видов архаров во всем их генетическом многообразии. Одним из перспективных методов сохранения генофонда исчезающих видов диких животных является метод глубокого замораживания сперматозоидов и трансплантации эмбрионов.

Вид архар или горный баран включает в себя несколько достаточно хорошо изученных и отличающихся по внешним характеристикам подвидов:

- алтайский баран или Ovis ammon ammon;
- анатолийский муфлон или Ovis ammon anatolica;
- бухарский баран или Ovis ammon bocharensis;
- казахстанский аргали или Ovis ammon collium;
- ганьсуйский аргали или Ovis ammon dalailamae;
- тибетский баран горный или Ovis ammon hodgsonii;
- северокитайский баран горный или Ovis ammon jubata;
- тяньшанский баран горный или Ovis ammon karelini;
- аргали Козлова или Ovis ammon kozlovi;
- каратауский баран горный или Ovis ammon nigrimontana;
- кипрский баран или Ovis ammon ophion;
- баран горный Марко Поло или Ovis ammon polii;
- кызылкумский баран горный или Ovis ammon severtzovi;
- урмийский муфлон или Ovis ammon urmiana.

Сохранению биологического разнообразия во всем мире заслуженно уделяется большое внимание. Проблема ускоренного воспроизводства генетических ресурсов приобрела особую актуальность в настоящее время, когда Красная книга ежегодно становится объемнее, а окружающий нас мир — все беднее. Ежегодно планета теряет множество видов животных и растений. Поэтому изыскание возможностей сохранения, находящихся на грани исчезновения популяции диких животных имеет не только научный, но и большой практический интерес.

Теоретические основы сохранения редких и исчезающих видов диких животных Казахстана до сих пор не разработаны и еще недостаточно изучены. Применение биотехнологических приемов дают возможность сохранению генетической основы ценных и исчезающих видов животных.

Ключевые слова: индукция, полиовуляция, синхронизация, овцематка-реципиент, эмбрион, инъекция, осеменение, трансплантация, архары.

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