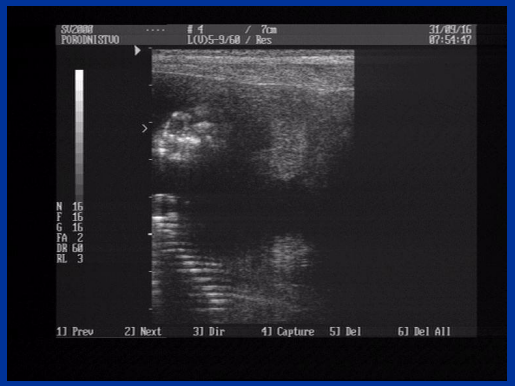

Current state, challenges and perspective of cryopreservation for croatian autochtonous breeds of domestic animals-croatian experience of sheep breeds

***N. Prvanović Babić*, M. Lojkic, S. Vince, N. Macesic,,**
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B. Spoljaric, I. Folnozic
,
Faculty of veterinary medicine
University of Zagreb
Croatia

Cryopreservation-ART (assisted reproduction tehniques)

Laboratory animals (rodents, rabbits)

Commerical breeds

Research/goverment/university labs

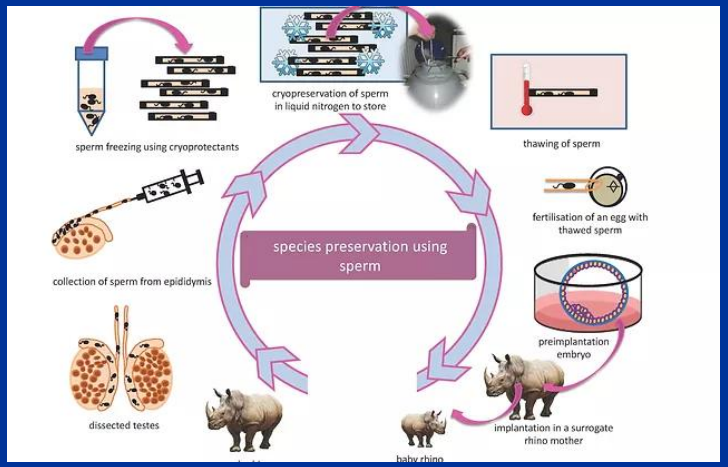
Commercial labs/global trade

Gene bank dependent on all numbered

Natures safe-global network

NATURE'S SAFE

SAVING ANIMALS FROM EXTINCTION



Global trends of ART in research and breeding

Cryopreservation as a method of eradication of sexually transmitted deseases

Embryotransfer as a method for increasing genetic superiority in dairy industry

ICSI and embryotransfer as a method of choice for breeding of superior subfertile sport mares

Cloning as a method of obtaining offsprings from genetically superior geldings (horses and dogs) and pets

Croatian trends in ART research and breeding

State versus commercial owned centers for reproduction due to EU membership

Global market

EU legislation

Project/research funding

Registered/approved labs

Global market

Import/export

On line auctions

Genomic selection

Animal (2018), **12:S1**, pp s4–s18 © The Animal Consortium 2018
doi:10.1017/S1751731111800071X

animal

Review: Historical and futuristic developments in bovine semen technology

P. Lonergan†

School of Agriculture and Food Science, University College Dublin, Dublin, D04N2E5, Ireland

Journal of Equine Veterinary Science 32 (2012) 451-454

Journal of Equine Veterinary Science

journal homepage: www.j-evs.com

Review Article

How Stallion Sperm Age In Vitro? Scenario for Preservation Technologies

Fernando J. Peña DVM, PhD, Dipl. ECAR[a], Cristina Ortega Ferrusola DVM, PhD[a], Jose A. Tapia DVM, PhD[b], Ines M. Aparicio PhD[b]

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ANNUAL REVIEWS

Annual Review of Animal Biosciences

Implications of Assisted Reproductive Technologies for Pregnancy Outcomes in Mammals

Peter J. Hansen

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ScienceDirect

Theriogenology 69 (2008) 23–30

Theriogenology

www.theriojournal.com

The mare model for follicular maturation and reproductive aging in the woman

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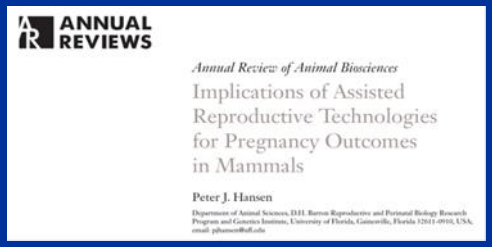
Table 1 Global statistics on number of reported transfers of embryos in domestic animal species[a]

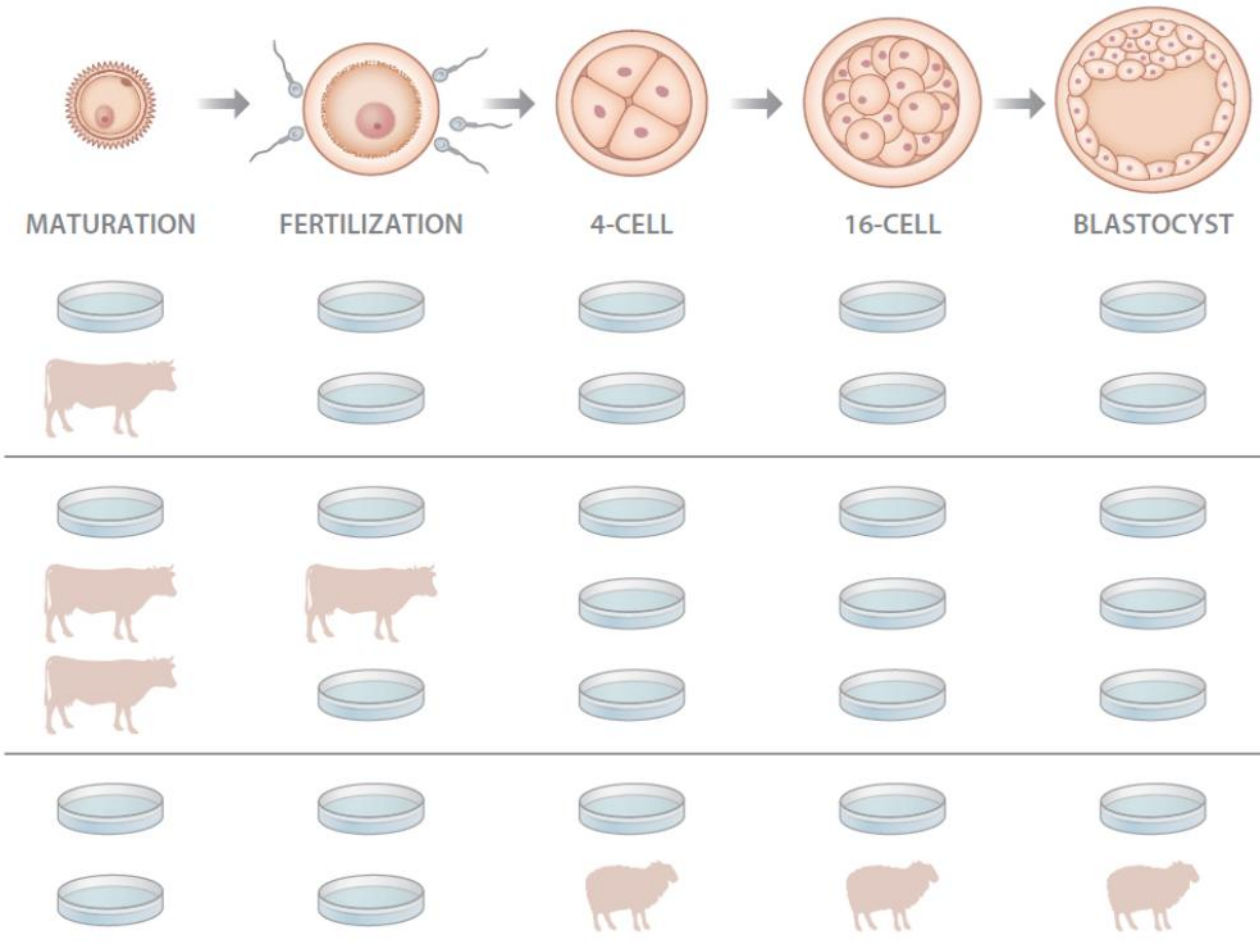
Species	Produced in vivo	Produced in vitro	Total
Cattle	406,287	757,652	1,163,939
Horse	20,468	543	21,011
Sheep	12,571	55	12,626
Goat	3,589	0	3,589

[a]Data are from Reference 61.

Table 2 Consequences of embryo culture on survival after transfer into recipient females

Species	Outcome	Result, % (no embryos transferred)		Notes	Reference
		In vivo	In vitro		
Cow (*Bos indicus*)	Pregnant at day 60	42% (289)	34% (9,210)	Same donors used for both procedures	82
Cow (*Bos taurus*)	Pregnant at day 41	49% (867)	39% (7,428)	Commercial conditions—recipients not randomly assigned	83
Sheep	Conceptus at day 17	80% (37)	53% (38)		85
Sheep	Fetus at day 42	75% (20)	33% (70)	Two embryos per recipient	84
Mouse	Live fetus at day 15	58% (70)	31% (82)	Morulae transferred	87

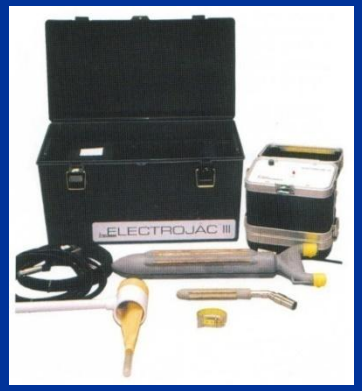


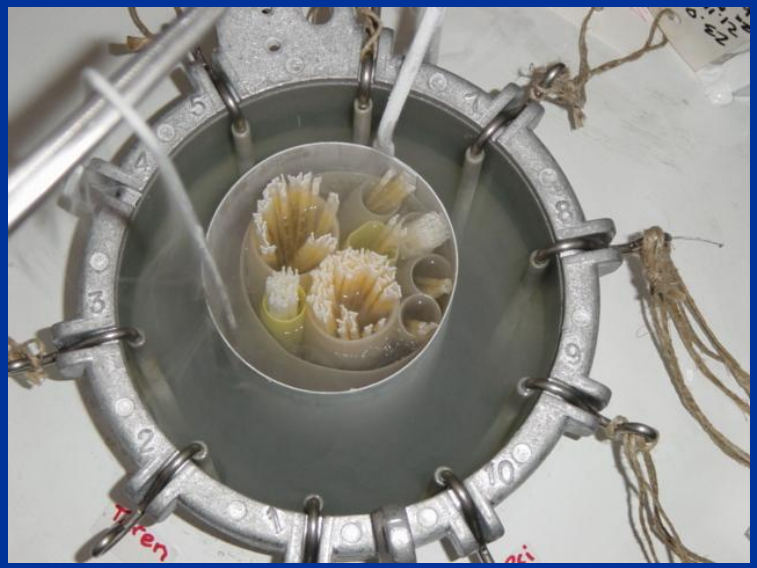


Percent blastocysts	Survival to vitrification
39	Poor
78	Poor
39	Poor
74	Poor
58	Poor
34	Poor
35	Good

Results of an experiment to evaluate consequences of in vitro maturation, fertilization, and development on percent of oocytes becoming blastocysts at day 8 of development and of the blastocysts surviving vitrification. Data are from Rizos et al. (118). Petri dishes indicate periods of culture, and animal icons indicate periods of development in vivo, in either heifers or sheep.

Getz i sur. (2004.): ART as preservation of endengered wild species and autochtonous breeds of ruminants *Zbornik radova Trećeg hrvatskog veterinarskog kongresa* , Opatija.





Frozen semen in gene bank

-buša

-slavonsko srijemski podolac

-boškarin

-istarska pramenka

Laparoscopic AI of sheep, MOET in cattle

Petrić i sur., 2001: Duboko smrzavanje sperme ovnova - primjena za intrauterino osjemenjivanje. *Zbornik radova Veterinarskih dana 2001, Opatija, listopad 2001.*

First calf after MOET (mulitiple ovulation, fertilisation in vitro, embryotransfer born in Croatia in 1995
First piglet born after AI in 1985
Flushing, freezing and ET of wild and domestic ruminants, frozen semen of dogs and horses

Measuring the conception rate via embryo recovery from uteruses of brown bears after embryonic diapause

Nikica PRVANOVIĆ BABIĆ[1], Slaven RELJIĆ[1], Relja BECK[2], Marina HABAZIN[1], Đuro HUBER[1]

[1]Faculty of Veterinary Medicine, Zagreb, Croatia [1]Croatian Veterinary Institute, Zagreb, Croatia

Introduction

- The fertilized ovum remains free-floating in the uterus (embryonic diapause) for up to five months
- The "real" pregnancy begins by attaching to the uterine wall at the beginning of hibernation
- Reproductive cycle of brown bear is slow with exact effects on ultimate reproductive success rate is still unknown
- Major processes of the reproductive cycle include ovulation, implantation, fetus development, parturition and neonate nurturing

Question to be investigated: **which of these processes has the greatest effect on the success of the whole cycle.**

- In majority of domestic mammals, major cause of reproductive failure is early embryonic mortality with a mortality rate up to 40% of fertilized eggs.

Materials & Methods

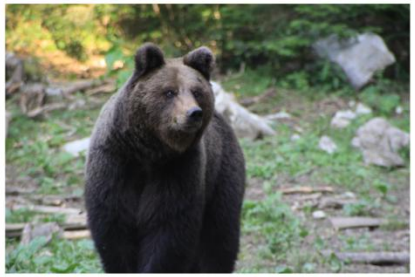


Figure 1. Female brown bear (*Photo S.R.*)

- We examined uteri of 4 adult females immediately after being shot in legal hunting
- The uteri were flushed with 60 ml of Ringer lactate solution and embryos were microscopically searched in Petri dish
- Presence of corpora lutea (CLs) has been searched on ovaries as indicator of mating and ovulation, and compared with number of recovered embryos
- Study was performed in November 2017 when implantation and active development of conceptus should begin since active fetal growth in bear lasts for 60 days and parturition in den is usually in January

Results

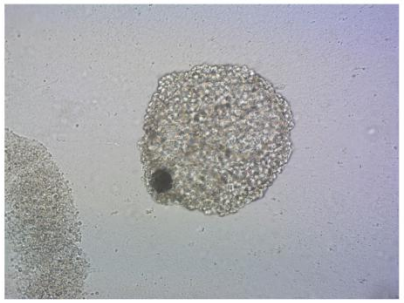


Figure 2. One of embryos obtained by flushing of uterus

- One female had 3 CLs and 3 embryos, two had 2 CLs and 2 embryos each, while one had one CL and one embryo; 8 embryos and 8 CLs in total
- All embryos were active with significantly more than 200 cells per embryo (activated stage, Figure 2.)

Mating rate 100%

Conception rate and embryo survival 100%

There was no proof of any embryonic mortality in observed bears after Embryonic diapause stage, since number of recovered embryos corresponded with number of CLs

26th INTERNATIONAL CONFERENCE ON BEAR RESEARCH & MANAGEMENT
16th - 21st September 2018, Ljubljana, Slovenia

Acknowledgements:
This study was supported by the project LIFE DINALP BEAR (LIFE13 NAT/SI/000550) and the European Commission under the "HUNT" project of the 7th Framework Programme for Research and Technological Development.

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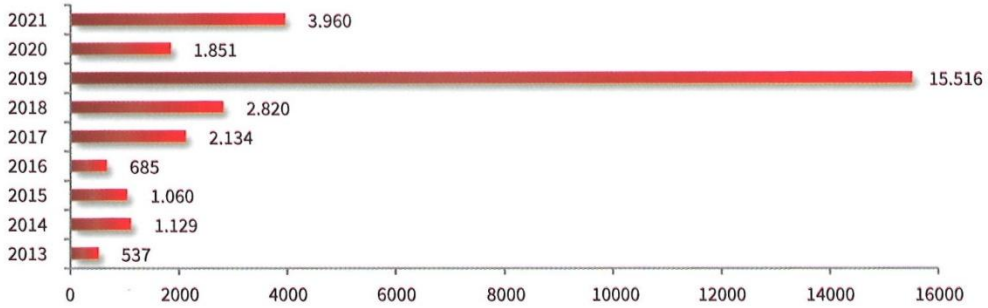
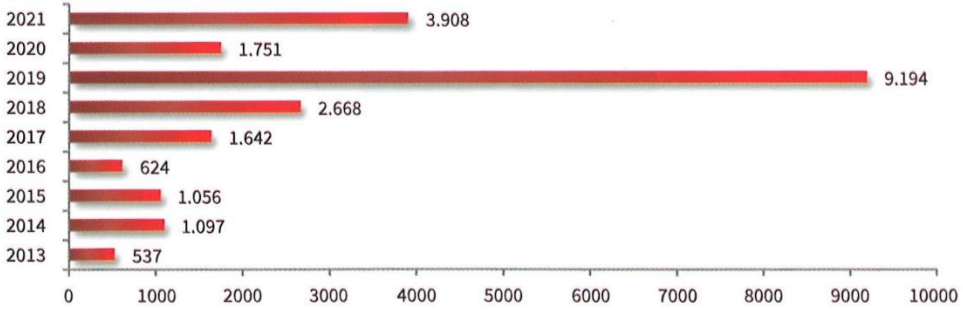
Tablica 1. Genomska kolekcija Banke gena domaćih životinja Republike Hrvatske

Broj vrsta	Broj pasmina	Broj izvornih pasmina	Broj uzoraka izvornih pasmina	Broj uzoraka 2013-2021
6	43	25	22.477	29.692

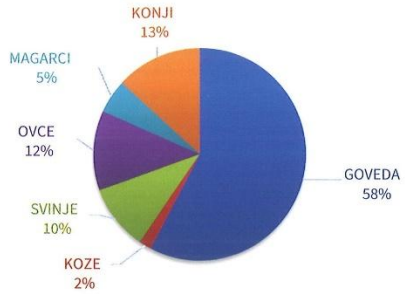
Grafikon 1. Pregled pohranjenih bioloških uzoraka u Banci gena domaćih životinja u periodu od 2013. do 2021. godine



Grafikon 2. Pregled pohranjenih bioloških uzoraka izvornih i ugroženih pasmina u Banci gena domaćih životinja u periodu od 2013. do 2021. godine



Udio pohranjenih uzoraka prema vrsti domaćih životinja prikazani su u grafikonu 3. i tablici 2., a prema vrsti uzorka u grafikonu 4.

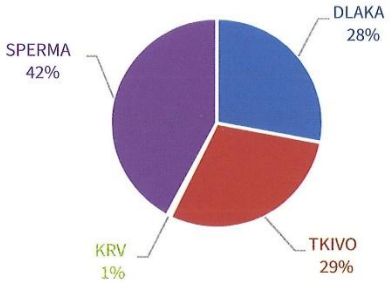


Grafikon 3. Udio pohranjenih bioloških uzoraka u Banci gena domaćih životinja prema vrsti domaćih životinja

Tablica 2. Udio pohranjenih bioloških uzoraka u Nacionalnoj banci gena domaćih životinja prema vrsti domaćih životinja u periodu od 2013. do 2021. godine

Vrsta životinja	Broj pohranjenih uzoraka	Udio pohranjenih uzoraka (%)
Goveda	17.126	57,7
Svinje	2.968	10,0
Ovce	3.603	12,1
Koze	562	1,9
Konji	3.906	13,2
Magarci	1.527	5,1
	29.692	100,0

Grafikon 4. Udio pohranjenih bioloških uzoraka u Nacionalnoj banci gena domaćih životinja prema vrsti uzorka



Tablica 3. Pregled pohranjenih bioloških uzoraka u Nacionalnoj banci gena domaćih životinja prema vrsti domaćih životinja

Vrsta i pasmina	Dlaka	Tkivo	Krv	Sperma	Broj uzoraka	Broj jedinki
Govedo / pasmine						
buša	885	1.943	41	0	2.869	2.089
istarsko govedo	551	559	0	2.679	3.789	963
slavonsko srijemski podolac	168	150	67	3.666	4.051	259
ostale pasmine	88	175	0	6.154	6.417	363
Ovce / pasmine						
cigaja	32	115	0	0	147	147
creska ovca	0	84	0	0	84	81
dalmatinska pramenka	160	1.691	0	0	1.851	1.818
dubrovačka ovca - ruda	0	310	0	0	310	310
istarska ovca	134	162	0	0	296	238
krčka ovca	0	58	0	0	58	58
lička pramenka	29	461	0	0	490	471
paška ovca	0	215	0	0	215	209
rapska ovca	0	152	0	0	152	151
Koze / pasmine						
hrvatska bijela koza	2	21	0	0	23	23
hrvatska šarena koza	13	472	0	0	485	468
istarska koza	5	2	0	0	7	7
ostale pasmine	0	7	0	40	47	11
Svinje / pasmine						
turopoljska svinja	83	172	61	0	316	204
crna slavonska	554	1.522	0	0	2.076	1.729
banijska šara svinja	82	211	0	0	293	172
ostale pasmine	86	197	0	0	283	211
Konji / pasmine						
lipicanac	1.518	0	0	0	1.518	1.513
međimurski konj	27	0	0	0	27	27
hrvatski hladnokrvnjak	1.176	0	0	0	1.176	1.176
hrvatski posavac	834	0	0	0	834	834
ostale pasmine konja	351	0	0	0	351	339
Magarci / pasmine						
primorsko-dinarski magarac	946	0	0	0	946	925
sjeverno-jadranski magarac	43	0	0	0	43	43
istarski magarac	457	0	0	0	457	447
ostale pasmine magaraca	81	0	0	0	81	81
Ukupno (n (%))	**8.305 (28 %)**	**8.679 (29 %)**	**169 (1 %)**	**12.539 (42 %)**	**29.692**	**15.403**

Izvorne pasmine prema županiji: Istarsko govedo

	2010. godina			2019. godina		
ŽUPANIJA	Stada	Krave	Bikovi	Stada	Krave	Bikovi
Istarska	142	550	20	120	707	39
Ličko-senjska	1	5	1	15	118	4
Primorsko-goranska	5	44	2	9	96	0
Splitsko-dalmatinska	0	0	0	2	22	3
Koprivničko-križevačka	1	0	1	3	18	1
Sisačko-moslavačka	1	0	0	1	14	1
Zadarska	0	0	0	1	9	1
Karlovačka	0	0	0	2	5	1
UKUPNO	**150**	**599**	**24**	**153**	**989**	**50**

Izvor: Godišnje izvješće HPA 2010 Govedarstvo.; HAPIH, Godišnje izvješće, 2019.

Broj grla izvornih i ugroženih pasmina (Istra):

	2009.	2013.	2015.	2016.	2017.	2018.	2019.
Istarsko govedo	531 *IA*	761 *I*	834 *I*	865 *I*	909 *I*	969 *I*	1039 *I*
Istarska ovca	2142 *II*	2871 *II*	1943 *II*	1245 *I*	1589 *II*	1484 *II*	1632 *II*
Istarska koza	? *	36 *IA*	24 *IA*	29 *IA*	36 *IA*	38 *IA*	39 *IA*
Istarski magarac		188 *IA*	490 *IA*	490 *IA*	548 *IA*	600 *IA*	677 *IA*

Status ugroženosti:

II – potentially endengered

I – highly endengered

IA – critically endengered

Annual reports of Croatian Ministry for agriculture

- **mađarski sivi podolac**
- populacija se tijekom 70-tih godina prošlog stoljeća svela na svega 187 ženki i 6 mužjaka.

Program zaštite mađarske vlade: Embriotransfer, zamrzavanje sjemena i zametaka
Pasmina spašena od izumiranja i sada broji oko 1000 jedinki (*Solti i sur.*, 2000).

Nacionalni park Hortobágy pusta

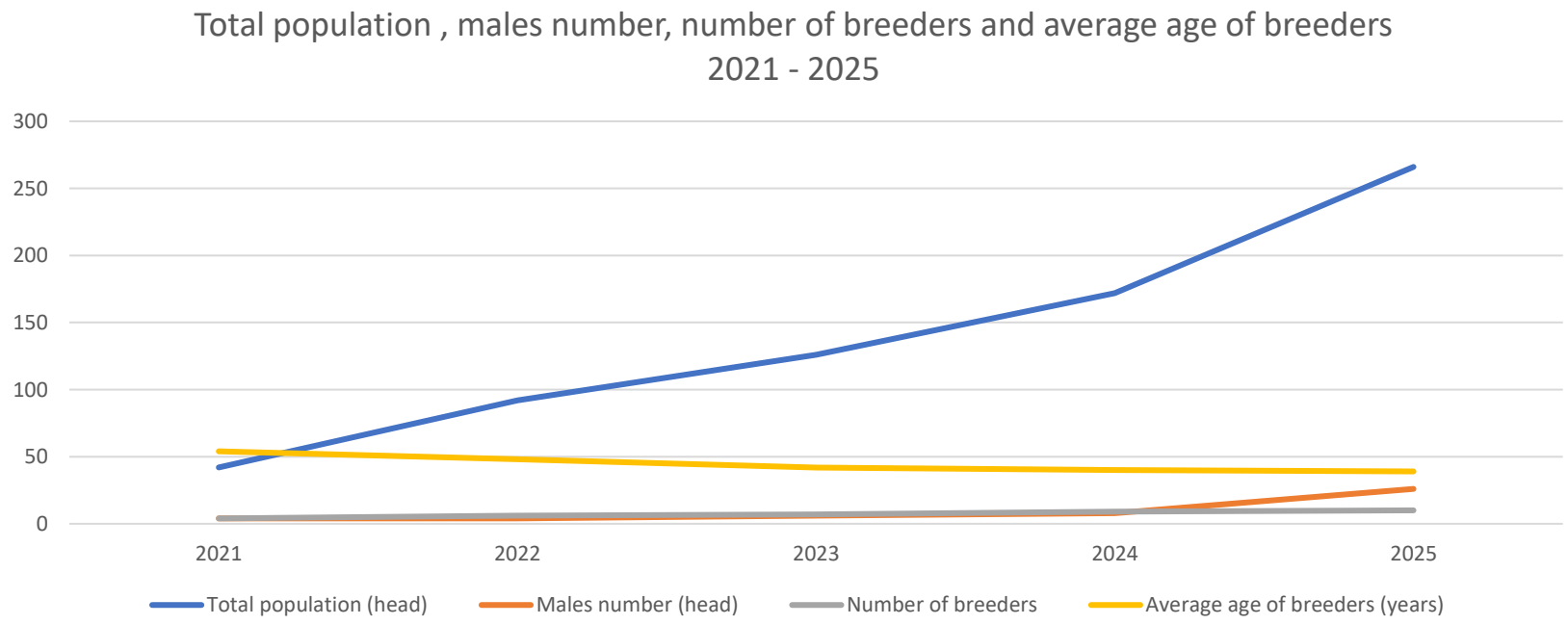
Action plan for the conservation and valorisation of the Istrian goat (2021, Region of Istria)

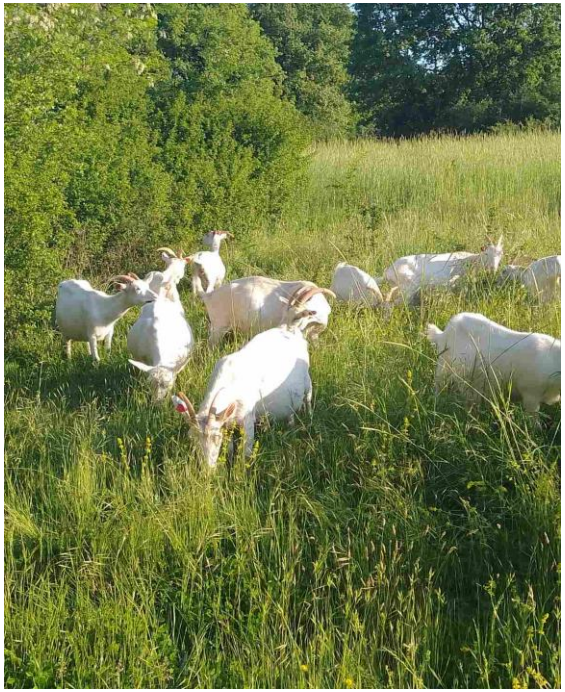
- Why? – Coat of arms of Istria, Croatia, **landscape management**, extensive breeding, milk and meat production
- When? – 2012. verified as a Croatian autochthonous breed
- 1947 – 1990. – Prohibited breeding of goats, just a few goats on farm
- 2020. only 42 goats remain
- Holder of Action Plan: Region of Istria
- Action Plan Implementer: AZRRI – Agency for Rural Development of Istria

ACTION PLAN PARTS

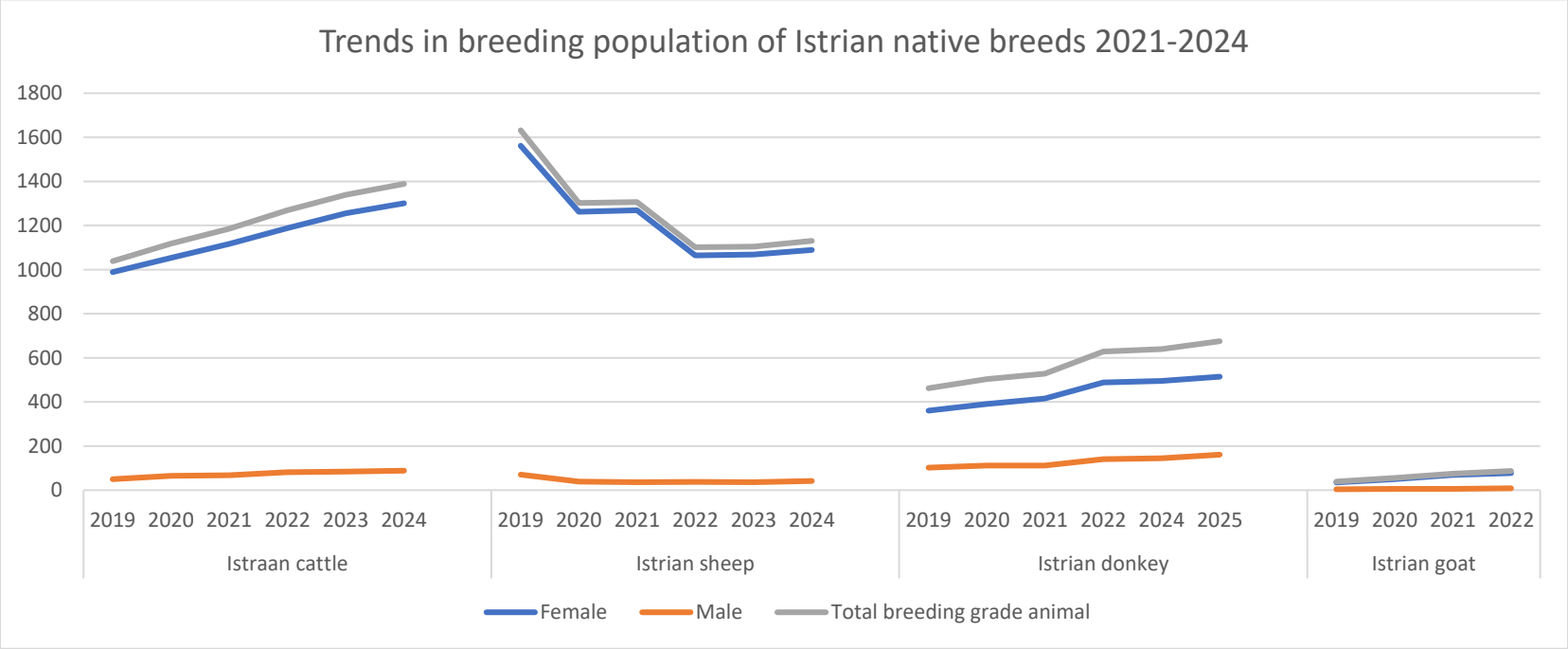
Breeding Programme	Genetic Resources	Grants	Promotion	Valorisation of Products
• **establishing new herds and increasing herd size - every breeding-grade female animal kept in breeding** • **scientific research on productivity and genetic**	• AZRRI Animal Gene bank – whole breeding-grade population genotyped (65K) 2021, 2022, 2023,2024… • 2026. The Veterinary Faculty of the University of Zagreb starts the cryopreservation program	• EU grants for endangered autochthonous breeds • Istrian Region's Grants for increasing herd size	• Funding „OUR ISTRIAN GOAT“: the possibility of adoption of the goat • Promotion of the breed in the media • Cheesemaker training	• Educational gastronomic centre of Istria – promotion and workshops from chefs to chefs • Promotion of the environmental services of goats

Results:


Total population , males number, number of breeders and average age of breeders
2021 - 2025
300
250
200
150
100
50
0
2021
2022
2023
2024
2025
Total population (head)
Males number (head)
Number of breeders
Average age of breeders (years)



Results: trends in indigenous breed populations supported by multidisciplinary support (Program for Istrian sheep started in 2024)



Further goals:

- Network of education and collaboration between breeders and experts
- Decrease of infertility, abortions and neonatal losses
- Increase of number of topic lambs (endengered lines and sires)
- Better organisation of matings and better control under reproduction of croatian autochtonous breeds
- Increased number of field experts for reproductive problems in sheep and increased total amount of knowledge about sexually transmited deseases and assisted reproduction as a tool of help

LIFE DINALP BEAR

LIFE13 NAT/SI/000550

For optimal results:

Cryopreservation is excellent tool for overcoming time and distance on short term

Cryopreservation is not substitute for preservation invivo

Further research is needed to detect and overcome species and breed differences in capablity for ART usage

Networking and collaboration on regional and global level are key to success

Education of breeders and sensibilisation of global audience, esspecially children are a must

I am open for questions and further collaboration

My contact adress:

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