ELABORATION OF A NOVEL MODEL FOR THE REINTEGRATION OF AVIAN PGCS BY THE CREATION OF A UNIVERSAL STERILE RECIPIENT

Mariann Molnár
Gödöllő
2024

The PhD program:

Name:	Doctoral School of Animal Biotechnology and Animal Science
Discipline:	animal husbandry
Leader of the school:	Prof. Dr. Miklós Mézes Member of the HAS, Head of Department Hungarian University of Agriculture and Life Sciences, Institute of Physiology and Animal Nutrition, Department of Nutritional Safety
Supervisor:	Dr. Eszter Várkonyi Senior Research Fellow National Centre for Biodiversity and Gene Conservation, Institute for Farm Animal Conservation

.....
Approval of the head of the doctoral school
Prof. Dr. Miklós Mézes

.....
Approval of the Supervisor
Dr. Eszter Várkonyi

1. BACKGROUND AND OBJECTIVES OF THE RESEARCH

Protection of rare or endangered species is important not only for wild, but also for livestock species that have cultural and scientific values but receive less attention. Many species excluded from everyday farming practices and threatened with extinction without genetic conservation programs. In the case of several mammalian species, protocols are available for the conservation of different genetic material, but most of these technologies are not or only partially adaptable to birds due to their significantly different reproductive biology. Semen freezing technology is already used in the case of many avian species and breeds, but it does not ensure the preservation of the entire genetic material, it also requires the safe conservation of the female sex, which is a particular challenge because of the large size and specific characteristics of the egg. Avian gene preservation research focus on the collection, safe preservation and potential use of the early embryonic stem cells and gonadal tissues of 1-day-old hatchlings. After the injection of donor derived primordial germ cells (PGCs) into surrogate host animal, the cells can colonize the embryonic gonad and this chimeric bird will be able to produce donor derived gametes. During this procedure, it is always a problem that the recipient individual has not only donor derived gametes, but also contains its own germ cells that greatly affect the efficiency of the transplantation. There are several methods to inhibit the formation of gametes in the recipient; however, these procedures involve high risk regarding the viability of the embryo. Gene editing, which provides precise and fast results, may encounter legal obstacles in many countries, therefore the present experiments approach the achievement of recipient sterility from an alternative direction: to create a sterile interspecific hybrid host by crossing Guinea fowl and domestic fowl species and test its suitability to receive donor PGCs.

The main objectives of the experiments:

- production of interspecific hybrids by crossing Guinea fowl hens x domestic fowl cockerels and Guinea fowl cocks x domestic fowl hens
- to investigate the sterility and suitability to receive donor PGCs of the produced hybrids
- testing the suitability of the hybrids by injection of GFP-labelled PGCs
- to investigate the presence of injected donor-derived PGCs in the gonads by histopathological and immunohistochemical examinations at embryonic age and in mature individuals

2. MATERIALS AND METHODS

2.1. Production of hybrids

Hybrids were produced by crossing of Yellow Hungarian Chicken (*Gallus gallus domesticus*) and Hungarian Landrace Guinea fowl (*Numida meleagris*). Guinea fowl females were artificially inseminated with sperm of Hungarian Yellow cockerels and during the reverse crossing, Hungarian Yellow hens were inseminated with sperm of Guinea fowl males (Bakst & Dymond, 2013). Eggs were collected twice a day and stored 15-17°C until use. Incubation was performed with 45° rotation every two hours, at 37.8°C temperature, with 70% of relative humidity. During hatching, the hatching time, the developmental stage of dead embryos and the incidence of chromosomal abnormalities were described.

2.2. Preliminary investigations of the ability of Guinea fowl x domestic fowl hybrids to receive donor delivered primordial germ cells

2.2.1. Direct chromosome preparation of dead embryos

Embryonic tissues were collected from died embryos during incubation and samples from chromosome preparations were investigated under light microscope in order to define the sex of the individuals and the incidence of chromosomal abnormalities.

2.2.2. Investigation of embryonic gonads with immunostaining

Gonadal tissues were collected from 6-, 10- and 20-days-old embryos during incubation of hybrid eggs from crossing of domestic fowl males and Guinea fowl females. In order to investigate whether the gonads of hybrids contain primordial germ cells, prepared sections were immunostained and slides were examined under fluorescent microscope.

2.2.3. Verifying hybrid status of offspring with molecular genetic markers

To verify the hybrid status of the hatched offspring, DNA was isolated using a salting-out method (Miller *et al*. 1988) modified for poultry species (Bodzsar *et al.* 2009) from blood samples of Guinea fowls, domestic fowls and phenotypically hybrid individuals. For the polymerase chain reaction, tailed primers were used with different fluorescent labels (GUJ1, GUJ87). Data was analysed with fragment analysis software that identified the allele sizes for the microsatellite markers of both species and this made possible to define the hybrid status of the examined individuals.

2.2.4. Investigation of gonads of the raised hybrids

To investigate the gonads of hatched hybrids, the individuals were raised to maturity and in every two weeks between the 16th and 30th week of growth 4–5 hybrids and one or two control Guinea fowls were sacrificed for histological analysis. Blood, liver and muscle tissue samples were collected for molecular genetic analysis. Histological sections were made from fixed and embedded tissues in paraffin, then hematoxylin-eosin staining was performed and the slides were covered for microscopic examination at x100 magnification.

2.2.5. In vitro investigation of fertilizing ability

Because of poor fertility of eggs during crossing of Hungarian Yellow hens and Guinea fowl males, *in vitro* test was performed in order to investigate the rate of guinea fowl spermatozoa that were able to pass up the oviduct and reach the oocytes of domestic hens after insemination. After opening the eggs, a 1×1 cm piece of the perivitelline membrane from over the germinal disc was cut around and after washing, the piece of membrane was spread on a microscope slide. The total numbers of the hydrolysed holes produced by

spermatozoa were counted with a ×4 objective using dark field optics. (Staines *et al.* 1998)

Another *in vitro* test was performed to investigate the ability of guinea fowl spermatozoa to penetrate the perivitelline membrane in chicken (infertile) table eggs. A 0,5 x 0,5 cm piece of the perivitelline membrane on the opposite side from the germinal disc was cutted and washed, then incubated with slow mixing at 38°C for 5 minutes with 1 ml DMEM solution that contained 25 million guinea fowl spermatozoa. After that, the piece of membrane was spread on a microscope slide and 3 fields of view were examined with a ×4 objective using dark field optics (Steel *et al.* 1994).

2.3. Injection of GFP-expressing PGC lines into 3-day-old hybrid embryos

As the results of the preliminary studies showed that only crosses between Guinea fowl hens and domestic fowl cockerels were suitable for the production of hybrids, further tests were performed to investigate the suitability of hybrids to receive donor PGCs: GFP-expressing PGCs were injected into the bloodstream of 3-day-old hybrid embryos and the incorporation of donor cells into the gonads was examined at embryonic age and in mature individuals.

2.3.1. Phenotypic analysis of dead embryos

The injected eggs which did not hatched were opened and the day of the death, the developmental abnormalities were recorded in order to compare the results with the non-injected control eggs and define the effects of injection on hatchability.

2.3.2. Observation of injected GFP-labelled PGCs in the embryonic gonads

Integration of GFP-expressing PGCs into the gonads was examined in 7.5, 14.5 and 18.5 days-old embryos. After dissection, the gonads were fixed in 4%

paraformaldehyde solution for a night, then stored in PBS and examined under fluorescence microscope.

In the case of 18.5 days old embryo, after fixation, the gonads were embedded in gelatine blocks and cross-sections were immunostained to examine the integration of the injected PGCs.

2.3.3. Examination of the gonads of mature hybrid individuals

Gonads of two injected and one control individuals were examined by immunostaining and histological sections were made in order to investigate the integration of the injected cells.

After fixation, the gonads were embedded in gelatine block, cryosectioned and on one part of the cross-sections haematoxylin-eosin staining was performed. The covered slides were examined with light microscope at x100 magnification.

On the other parts of the slides DAPI staining were performed and the GFP-expression was examined under confocal microscope.

3. RESULTS AND DISCUSSION

3.1. Production of hybrids

44.35% of hybrid eggs produced by crossing of Guinea fowl hens with Hungarian Yellow cockerel were infertile and 20.26% underwent an early embryonic death. 11.62% of developing embryos died within 5 days of incubation, 1.56% between the 6th and 10th day, 0.78 % between the 11th and 15th day, 1.23% between the 16th and 20th day, 1.30% between day 21 and 27. The rate of perinatally dead embryos was 11.17% and **6.95%** were hatched. 0.78 % of dead embryos had abnormalities such as cranial malformations, abdominal wall defects, beak deformities and dwarfism. In case of hybrid embryos, an extended period of hatching time was experienced (from day 21 to 27) which is a transition between the Yellow Hungarian (21-22 days) and the Guinea fowl (26-29 days). Most of the hybrids (57.9%) hatched on the 22-23rd day.

Crossing of domestic fowl hens and Guinea fowl males was unsuccessful. 98.43% of 702 incubated eggs was unfertile, 1.13% underwent an early embryonic death, 0.14% of developing embryos died within 16th and 20th days of incubation. The rate of perinatally dead embryos was 0.14% and only one chick hatched (**0.14%**).

3.2. Results of preliminary investigations of the ability of Guinea fowl x domestic fowl hybrids hosting the donor delivered primordial germ cells

3.2.1. Direct chromosome preparation of dead embryos

Chromosomal analyses of 187 samples were performed. Based on these results, the proportion of males was **56.76%,** and the females were **43.24%**. Two types of chromosomal abnormalities were observed during the

investigations: aneuploidy and mosaicism. The ratio of specimens with aneuploid chromosome abnormality was 0.9%. The proportion of haploid/diploid (1n/2n) mosaic karyotypes was 1.8%.

During the investigation of seven samples from reverse crossing (domestic fowl hen x Guinea fowl male), three of them were evaluated. Two female and one male were detected and chromosomal abnormalities were not observed.

3.2.2. Investigation of embryonic gonads with immunostaining

Out of the eight embryonic samples examined, four were females based on gonadal structure. During this investigation, two markers were used to detect germ cells in hybrids: the stem cell-specific SSEA-1, which is reliable from stage X (Eyal-Gilaldi & Kochav 1976), and the germ cell-specific marker p63. At the female samples many germ cells show co-staining of p63 and SSEA1. The germ cells are distributed throughout the gonad, not concentrated in the cortex. The p63 positive cells are circularly located along the seminal vesicles in male samples. It can be concluded that germ cells are present in the gonads of hybrids of both sexes.

3.2.3. Verifying hybrid status of offspring with molecular genetic markers

Result of fragment analysis to investigate the hybrid status of the individuals showed, that in case of the marker GUJ1: 260 bp, 262 bp and 264 bp allele sizes were observed in control chickens, while 241 bp and 243 bp allele sizes were detected in control Guinea fowl. In individuals which appeared to be hybrids, 260 bp or 264 bp and 243 bp size alleles were found. For marker GUJ87, the amplified allele sizes were 161 bp in chicken and 153 bp in Guinea fowl. Both alleles were detected in the putative hybrid individuals. Based on the microsatellite marker analysis, all of the 38 putative hybrid individuals were hybrids.

3.2.4. Investigation of gonads of the raised hybrids

During the investigation of the raised hybrids, it was observed that all individuals had two gonads, which were generally not at the same height and slightly inclined in shape. Between 16^{th} and 30^{th} weeks, their size did not change significantly. On histological sections of gonads from 16 weeks to 20 weeks old hybrids, signs of sperm formation, spermatocytes were not found, minimum proliferation activity was observed and inactive or infantile testicular cells could be observed. From week 22 to week 30, the hybrid testes samples displayed a typically normal tubular structure, however the cells are polymorphic. The germinal epithelium is not active; signs of spermiocytomorphogenesis were not visible.

In the case of Guinea fowls observed as controls, the testes of the males were fully developed and their size greatly exceeded the size of the hybrid gonads. The ovaries of females were also developed by week 16, and they began to lay eggs at week 26.

In contrast to the observation of embryonic gonads, it can be assumed that the examined 38 histological samples all originated from male individuals.

3.2.5. In vitro investigation of fertilizing ability

The results of the examination of eggs collected from the unsuccessful crossing combination (domestic fowl hen x Guinea fowl male) showed that in 37% of the eggs sperm penetration holes were not observed on the perivitellin membrane. In 42% of the cases, not above the germinal disc, but in the surrounding region a large number of uncharacteristic penetration holes were observed. The results suggest that guinea fowl spermatozoa are able to reach the location of fertilisation, but this crossing combination of the two species does not function due to the inhibition of the presumed chemical or other signalling pathway (chemotaxis) and the species-specificity of the sperm-binding receptors. In addition, the observed penetration holes are not as characteristic as

in normal cases, which could be due to the presumed inability of the acrosin of guinea fowl spermatozoa to properly dissolve the membrane structure.

The *in vitro* membrane test showed that only a very small number of guinea fowl spermatozoa were able to make a penetration hole in the perivitelline membrane of the hen egg.

3.3. Injection of GFP-expressing PGCs into 3-day-old hybrid embryos

3.3.1. Phenotypic analysis of dead injected embryos

57.85% injected embryos died within 5 days of incubation, among them, the rate of died embryos after the injection (on the 3rd day) means the 32% of all injected embryos. Proportion of died embryos between the 6th and 10th day of incubation was 21.85%, 5.54% died between the 11th and 15th days, 4.62% between the 16th and 20th day, 4.31% between day 21 and 27. Developmental abnormalities were observed in 2.77% of the embryos, which means in most cases abdominal wall defects and in some cases the deformation of the upper or/and lower beak. The rate of perinatally dead embryos was 1.54%, and **1.54%** of injected eggs hatched.

Comparing hatching data of non-injected control and injected hybrids, significant differences between the two groups were observed in the case of died embryos within the first 10 days of incubation, between the 11th and 15th days, the perinatally dead embryos and the hatched individuals. No significant difference was observed in the proportion of embryos that died between days 21 and 27 of incubation.

The high number of dead injected embryos in the first 10 days of incubation was presumably due to the injection procedure on day 3. The significant difference later in the incubation period (days 11-20) may be due to the fact that the injected eggs were incubated with a parafilm-covered window, which allows pathogens to enter and infect the developing embryo more easily. The reason for the high number of perinatal death in control group was that the hybrid chicken

had extreme difficulty in breaking through the hard Guinea fowl eggshell. It is difficult to decide during hatching, if and when human intervention is needed because of the prolonged hatching period (21-27 days). The hatching parameters of domestic fowl and Guinea fowl also differ significantly. During the incubation it was observed that hatching rate can be increased by changing parameters, such as increasing the humidity. In the case of injected individuals, the embryo can be assisted more efficiently due to the window, but the presence of the window may also affect the optimal incubation parameters of hybrid individuals.

3.3.2. Observation of injected PGCs in embryonic gonads

At 7.5 days of incubation, 15 out of 30 injected embryos were alive at the time of investigation and 12 of them contained integrated injected PGs. Another 75 embryos were examined at 14.5 days of age and 42 embryos at 18.5 days of age. To identify PGCs, CVH antibody was used, which expressing in cytoplasm throughout development. In every embryo alive at the time of the study, donor-derived PGCs were observed in the gonads.

3.3.3. Examination of the gonads of mature individuals

Similar to the control individual, the gonads of raised injected hybrids were different in size and length, show elongated, slightly bent shape. Compared to the development of male gonads in domestic fowl, the maturation of testes in hybrids is a much slower process.

On histological sections of one of the injected individuals at 46 weeks of age, spermatid cells in different stages of maturation and mature spermatozoa indicated by morphology were observed in seminiferous tubules. The results of immunohistochemical study showed that donor-derived GFP-expressing PGCs colonized almost 100% of recipient’s gonads. The sections showed that gametes at different stages of maturation in the gonads are of donor origin.

On histological sections of the other injected individual at 44 weeks of age, no mature spermatozoa were observed, but early maturing spermatids in the seminiferous tubules are visible. The immunostained sections show the donor-derived parts of the gonad.

The gonad of the control individual also shows a normal tubule structure, but gametes and their progenitors are not visible in the seminiferous tubules. In contrast to the injected individuals, only DAPI staining is visible on the section of control gonad.

To summarize the results, domestic fowl PGCs were transplanted successfully into sterile interspecific hybrid recipient embryos. The transplanted PGCs colonised the gonad of the host organism and developed into mature sperm.

4. CONCLUSIONS AND SUGGESTIONS

In connection with research aimed the long-term preservation of the female genome, the target of the described experiments was to create interspecific hybrids and test their suitability for receiving donor delivered primordial germ cells (PGCs).

Interspecific hybrids were produced by crossing of Yellow Hungarian chicken (*Gallus gallus domesticus*) and Hungarian Landrace Guinea fowl (*Numida meleagris*). Crossing of Guinea fowl females and domestic fowl males was successful; however, crossing of Guinea fowl males and domestic fowl females was unsuccessful, because the percentage of infertile eggs has extremely high. This infertility may be explained by the low number of guinea fowl spermatozoa which are able to reach the exact location of fertilisation and most of the created penetration holes are not in the right place (above the germinal disc). The signalling pathways (chemotaxis, activity of sperm-binding receptors, acrosome reaction) that help spermatozoa to find the germinal disc and are necessary for fusion between gametes are presumably not functional in this combination of crosses between the two species.

All the hybrids produced by the successful crossing (Guinea fowl female x domestic fowl male) were sterile males. Histological studies have shown that the hybrids do not produce endogenous sperm cells, but their gonads have normal tubular structure, suggesting that they may be able to receive PGCs from donor origin. Immunohistochemical investigations showed that at embryonic age, these sterile hybrids have endogenous germ cells, but they do not develop into sperm cells during maturation. The sterility of the male and unviability of female hybrids produced in this study follows Haldane's rule; which states that "when in the F1 offspring of two different animal races one sex is absent, rare or sterile, that sex is the heterozygous (or heterogametic) sex". In the case of Guinea fowl – domestic fowl hybrids, an extreme case of the Haldane's rule is

manifested, when the female sex (ZW) is completely absent and the male sex (ZZ) is sterile.

Investigation of the gonads suggests that sexual maturity in these hybrids is initiated much more slowly than in domestic fowls or in Guinea fowl. This could be due to a deficiency in hormonal regulation resulting from the hybrid status, for example the absence or under activation of Leydig's interstitial endocrine cells in the testes (Dimitriadis *et al*, 2015).

As all of the reared hybrids were male individuals, further studies are needed to investigate whether female (ZW) primordial germ cells can colonise the male (ZZ) gonads, integrate into them and develop viable spermatozoa from this genotype (ZW). According to previous studies, the appearance and transmission of female genotype is possible using male chimeras (Naito *et al*. 1999, Liu *et al*. 2017, Trefil *et al*. 2017, Xu *et al*. 2019, Marinovic *et al*. 2022, Blank *et al*. 2024, Park *et al*. 2020).

Based on the results of this study, it might be worthwhile to raise injected hybrids for even longer (more than 46 weeks) to obtain gametes from their gonads and to recover the donor genotype. In addition, the hormonal background required for reproductive activity can be investigated further and, depending on the results, hormone treatment of hybrids to stimulate reproduction and gamete production may be considered.

It might be worth to extend the studies to test the suitability of other hybrid combinations; for example pheasant x domestic fowl hybrids, as both sexes are reported in the references (Asmundson & Lorenz 1957).

5. NEW SCIENTIFIC RESULTS

1. I have verified using molecular genetic markers that Guinea fowl and domestic fowl have hybrids, of which only the males are viable.

2. I have found that the production of domestic fowl - Guinea fowl hybrids is only successful by crossing Guinea fowl females with domestic fowl males. Reverse crossing is inefficient, because guinea fowl spermatozoa are not able to locate and penetrate the domestic fowl germinal disc.

3. I demonstrated with immunohistochemical studies, that the Guinea fowl x domestic fowl hybrids have their own germ cells, but do not develop into gametes during maturation. I have confirmed by histopathology that the hybrids are sterile.

4. I concluded that the produced hybrids are suitable for receiving injected donor-derived PGCs and I have demonstrated by immunostaining that the gonads of the raised individuals contain different stages of progenitors and mature sperm cells derived from the injected donor PGCs.

6. REFERENCES

ASMUNDSON V.S., LORENZ F.W. (1957): Hybrids of Ring-Necked Pheasants, Turkeys, and Domestic Fowl. In: *Poultry Science*, Volume 36, Issue 6, 1957, 1323-1334. p. DOI: https://doi.org/10.3382/ps.0361323

BAKST M.R., J.S. DYMOND (2013): Artificial Insemination in Poultry. In: Lemma, A. (Szerk.): *Success in Artificial Insemination - Quality of Semen and Diagnostics Employed*. InTech, Jan. 09, 2013. DOI: http://dx.doi.org/10.5772/54918

BLANK M.H., GOISSIS M.D., MOTTA DE AVELAR AZEREDO R., SILVEIRA L.F., GARCIA PEREIRA R.J. (2024): Safeguarding genetic resources of curassow through cryopreservation and transplantation of post-mortem recovered testicular cells into recipient roosters. In: *Theriogenology Wild*, 2024, 100084, ISSN 2773-093X, DOI: https://doi.org/10.1016/j.therwi.2024.100084.

BODZSAR N., EDING H., REVAY T., HIDAS A., WEIGEND S. (2009): Genetic diversity of Hungarian indigenous chicken breeds based on microsatellite markers. In: *Animal Genetics*, 40: 516-523. DOI: https://doi.org/10.1111/j.1365-2052.2009.01876.x

DIMITRIADIS F., TSIAMPALI C., CHALIASOS N., TSOUNAPI P., TAKENAKA A., SOFIKITIS N. (2015): The Sertoli cell as the orchestra conductor of spermatogenesis: spermatogenic cells dance to the tune of testosterone. In: *Hormones* (Athens). 2015 Oct-Dec;14(4):479-503. DOI: 10.14310/horm.2002.1633.

EYAL-GILALDI H., KOCHAV S. (1976): From cleavage to primitive streak formation: a complementary normal table and a new look at the first stages of the development of the chick. 1. General morphology. In: *Dev. Biol*. 49: 321-337.

HALDANE J.B.S. (1922): Sex ratio and unisexual sterility in hybrid animals. In: *Journ. of Gen.* **12**, 101–109 (1922). DOI: https://doi.org/10.1007/BF02983075

LIU C., CHANG I-K., KHAZANEHDARI K.A., THOMAS S., VARGHESE P., BASKAR V., ALKHATIB R., LI W., KINNE J., MCGREW M.J., WERNERY U. (2017): Uniparental chicken offsprings derived from oogenesis of chicken primordial germ cells (ZZ). In: *Biol Reprod*. 2017 Mar 1;96(3):686-693. DOI: 10.1095/biolreprod.116.144253.

MARINOVIĆ Z., LUJIĆ J., SUŠNIK BAJEC S., DJURDJEVIČ I., SNOJ A., HOITSY GY., URBÁNYI B., HORVÁTH Á. (2022): Evaluation of triploid rainbow trout Oncorhynchus mykiss as a surrogate parent for brown trout Salmo trutta m. fario and grayling *Thymallus thymallus.* In:

Aquaculture Reports, Volume 24, 2022. 101163. ISSN 2352-5134. DOI: https://doi.org/10.1016/j.aqrep.2022.101163.

MILLER S.A., DYKES D.D., POLESKY H.F. (1988): A simple salting out procedure for extracting DNA from human nucleated cells. In: *Nucleic Acids Res* 1988;16:1215.

NAITO M., MATSUBARA Y., HARUMI T., TAGAMI T., KAGAMI H., SAKURAI M., KUWANA T. (1999): Differentiation of donor primordial germ cells into functional gametes in the gonads of mixed-sex germline chimaeric chickens produced by transfer of primordial germ cells isolated from embryonic blood. In: *J Reprod Fertil.* 1999 Nov;117(2):291-8. DOI: 10.1530/jrf.0.1170291.

PANDA S.K., MCGREW M.J. (2022): Genome editing of avian species: implications for animal use and welfare. In: *Laboratory Animals*. 2022;56(1):50-59. DOI:10.1177/0023677221998400

PARK K.J., JUNG K.M., KIM Y.M., LEE K.H., HAN J.Y. (2020): Production of germline chimeric quails by transplantation of cryopreserved testicular cells into developing embryos. In: *Theriogenology*. 2020 Oct 15;156:189-195. DOI: 10.1016/j.theriogenology.2020.06.027.

STAINES H.J., MIDDLETON R.C., LAUGHLIN K.F., WISHART G.J. (1998): Quantification of a sperm – egg interaction for estimating the mating efficiency of broiler breeder flocks. In: *Br. Poult. Sci.* 39: 273-277.

STEEL, M.G. MELDRUM W., BRILLARD J.P. AND WISHART G.J. (1994): The interaction of avian spermatozoa with the perivitelline layer in vitro and in vivo. In: *J. of Reprod. and Fertil.* 101: 599-603.

TREFIL P., AUMANN D., KOSLOVÁ A., MUCKSOVÁ J., BENEŠOVÁ B., KALINA J., WURMSER C., FRIES R., ELLEDER D., SCHUSSER B., HEJNAR J. (2017): Male fertility restored by transplanting primordial germ cells into testes: a new way towards efficient transgenesis in chicken. In: *Sci Rep*. 2017 Oct 27;7(1):14246. DOI: 10.1038/s41598-017-14475-w.

XU D., YOSHINO T., KONISHI J., YOSHIKAWA H., INO Y., YAZAWA R., DOS SANTOS NASSIF LACERDA S.M., DE FRANÇA L.R., TAKEUCHI Y. (2019): Germ cell-less hybrid fish: ideal recipient for spermatogonial transplantation for the rapid production of donor-derived sperm. In: *Biol Reprod*. 2019 Aug 1;101(2):492-500. DOI: 10.1093/biolre/ioz045.

7. RELEVANT SCIENTIFIC PUBLICATIONS

Molnár, M., Lázár, B., Sztán, N., Végi, B., Drobnyák, Á., Buda, K., Nagy, N., Szőcs, E., Fejszák, N., Liptói, K., Gócza, E., McGrew, M.J., Várkonyi, E.: Successful formation of sperm cells from transplanted primordial germ cells in sterile interspecific avian recipients. **SCIENTIFIC REPORTS**, (under review)

Molnár, M., Lázár, B., Sztán, N., Végi, B., Drobnyák, Á., Tóth, R., Liptói, K., Marosán, M., Gócza, E., Nandi, S., McGrew, M. J., Patakiné Várkonyi, E. (2019): Investigation of the Guinea fowl and domestic fowl hybrids as potential surrogate hosts for avian cryopreservation programmes. **SCIENTIFIC REPORTS**, https://doi.org/10.1038/s41598-019-50763-3. **D1, IF: 4.585**

Lázár, B**.**, **Molnár, M.**, Sztán, N., Végi, B., Drobnyák, Á., Tóth, R., Tokodyné Szabadi, N., McGrew, M. J., Gócza, E., Patakiné Várkonyi, E. (2021): Successful cryopreservation and regeneration of a Partridge coloured Hungarian native chicken breed using primordial germ cells. **POULTRY SCIENCE** https://doi.org/10.1016/j.psj.2021.101207 **D1, IF: 3.352**

Patakiné Várkonyi, E., **Molnár, M.**, Sztán, N., Váradi, É., Végi, B., Pusztai, P. (2016): Egy értékes hazai baromfifajtánk, a magyar parlagi gyöngytyúk (*Numida meleagris*) embrionális blasztodermasejtjeinek mélyhűtése génmegőrzés céljából/ Cryopreservation of embryonic blastodermal cells of a valuable domestic poultry breed, the Hungarian landrace guinea fowl (*Numida meleagris*) as a biodiversity preservation method **MAGYAR ÁLLATORVOSOK LAPJA** 138/11: pp. 673-680. **Q4, IF: 0.031**

Other publications on the subject of the thesis:

Molnár, M. (2018): The use of infertile interspecific hybrids for a novel model of PGC reintroduction applicable in gene preservation for poultry. In: *Innovative Management of Animal Genetic Resources Newsletter*, 2018/3. pp.3.