Animal Reproduction Science 213 (2020) 106280

Contents lists available at ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

Improvement of the application of gonadal tissue allotransplantation in the i*n vitro* conservation of chicken genetic lines

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ARTICLE INFO

Keywords:
Gonadal grafting
Donor-derived progeny
Chicken

ABSTRACT

In avian species, the surgical technique for ovarian allotransplantation has been developed for domestic chickens; however, not all genotypes can be effectively used as recipients. The aims of the present study were to ascertain donor/recipient combinations for production of offspring from frozen/thawed ovarian tissues. The development of the technique is important because domestic chicken offspring have only been produced from fresh (never frozen) ovarian and from frozen-thawed testicular tissues. Information obtained from evaluating genetic differences of intensively selected lines in which there was successful pairing was compared in the indigenous breeds. Results indicate donor/recipient combinations were created which could be effectively used for gonadal tissue allotransplantations. Gonadal tissues of Yellow, Speckled and Partridge-color Hungarian, Black and Speckled Transylvanian Naked Neck chicken breeds were allotransplanted into White Leghorn or Novogen White breeds for offspring production. The gonadal tissues of these indigenous breeds were cryopreserved using vitrification procedures. There was successful allografting of frozen/thawed gonadal tissues at a rate between 20 % and 100 % depending on the genotype and sex, and histological examination and microsatellite marker analysis provided evidence that the donor ovarian and testicular tissues had the capacity for producing gametes. The hens of Speckled Transylvanian Naked Neck/White Leghorn combination using frozen/thawed ovarian tissues were produced for progeny tests. Of these, 58 % produced eggs and 9.1 % produced donor-derived offspring, based on data for both feather color markers and genetic analysis.

1. Introduction

In avian species, the method most widely used for *in vitro* gene conservation has been semen cryopreservation, although more advanced techniques are available for the long-term storage of early embryonic cells (BCs, PGCs). In the last 2 decades, it has been reported that there can be use of gonadal tissue transfer for genetic conservation purposes (Song and Silversides, 2006; Kosenko, 2007; Liptoi et al., 2013; Liu, 2013). This may be an especially viable alternative in preserving the female genome, because the heterogametic oocyte cannot be frozen due to the large amount of yolk and its biophysical characteristics, thus, there has not been efficacious techniques available for female genetic conservation. In males, gonadal tissue transfer can be useful in specific cases, when

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https://doi.org/10.1016/j.anireprosci.2020.106280
Received 13 September 2019; Received in revised form 28 November 2019; Accepted 9 January 2020
Available online 10 January 2020

semen collection is not possible.

In domestic chickens, gonadal tissues of chicks 1 day post-hatching can be excised, cut into 2–4 pieces and allotransplanted into recipients that are 1 day post-hatching and from each tissue piece there will be a complete development of an entire gonad which has the capacity for gametogenesis even without the administration of immunosuppressant (Song and Silversides, 2007a, 2008b; Liptoi et al., 2013). The gonads of chicks 1 day post-hatching can be cryopreserved, because primary oocytes have a peripheral location and are developmentally quiescent. Tissues from all genotypes, however, are not equally suitable recipients. When there are appropriate donor/recipient combinations with use of unfrozen gonadal tissues, there is successful allografting and development of 70%–80% of the allografts that are allo-transferred, whereas for other recipients this ratio was minimal or zero. Tetra SL/Tetra SL, Plymouth/White Leghorn (WL) and WL/New Hampshire (NH) combinations have been used for successful pairing of tissues for allotransplants (Kosenko, 2007; Song and Silversides, 2006, 2007a, 2007b; Liptoi et al., 2013).

The primary objectives in the present study were to develop a method for identifying suitable recipients for donor indigenous Hungarian chicken breeds and to obtain offspring from gametes in frozen/thawed ovaries, because domestic chicken offspring have been produced only with ovarian tissues that have never been frozen and frozen testes (Song and Silversides, 2007a, 2008b; Liptoi et al., 2013).

2. Material and methods

All applied methods were approved by the Directorate of Food Safety and Animal Health of the Government Office of Pest County (License number: PE/EA197-4/2016) and by the Institutional Ethical Review Board. Unless otherwise stated, all chemical materials used were acquired from Sigma.

2.1. Selection of the donor/recipient combinations

The decisions for pairing of different genotypes were based on a large-scale analysis of genetic diversity in chickens (Bodzsar et al., 2012; EC project GLOBALDIV). This previous publication is a reporting of the genetic relationships of all indigenous Hungarian chicken breeds as well as the WL and NH breed. When there were NH/WL donor/recipient combinations used for gonadal tissue allotransplantation in earlier studies, a large percentage of the allografting attempts were successful. There, therefore, was assessment of gonadal allografts using indigenous Hungarian breeds that were similar to these genotypes in the present study. It was determined that the Yellow Hungarian (YH) breed was genetically similar to the NH breed and that the White Hungarian (WH) breed was genetically similar to the WL breed.

Thus, when evaluating the first tested combination, the YH was used to provide the donor tissue allografts and the WL was the recipient of these tissue allografts. Based on the results from this study, and considering the theory of genetic diversity, other recipients were selected from the commercial lines (summarized in Table 1). There were a total of 135 birds (85 females and 50 males) used in the study. The number of animals used in the different experiments is provided in Tables 2 and 3.

Recipient hens produced for the offspring tests were inseminated utilizing Partridge Color Hungarian (PCH) male semen. This breed has a recessive genetic control of feather color, therefore, utilizing this breed allows for there to be no effect of the genetics of this breed on the color of the offspring of the birds of the indigenous Hungarian breeds. Donor-derived progeny, therefore, can be selected based on their color marker.

Recipient-derived progeny ($n = 6$) were used for assessing whether ovariectomy by cauterization induced any degeneration in tissues surrounding the ovary (Table 5).

The NW egg layer hybrid end product birds were obtained from Prophyl Ltd., Mohács, Hungary, and all other birds used in the study were from the breeding stock of the Research Centre for Biodiversity and Gene Conservation, Gödöllő. The artificial incubation was performed locally, in the experimental hatchery. For gonadal tissue transfer, animals used as donors and recipients were hatched within the same 24 h time period.

2.2. Preparation of donor tissues

Gonadal tissues were removed from the donors that were euthanized by cervical dislocation immediately prior to tissue collection.

Table 1
Genotypes applied in the donor/recipient combinations.

Donor Indigenous Hungarian breeds	Recipient Commercial lines
Yellow Hungarian (YH)	White Hungarian (WH) White Leghorn (WL)
Black Transylvanian Naked Neck (BTN)	Novogen White (NW) White Leghorn (WL)
Partridge Color Hungarian (PCH)	White Leghorn (WL) Novogen White (NW)
Speckled Transylvanian Naked Neck (STN)	Novogen White (NW)
Speckled Hungarian (SH)	Novogen White (NW)

Table 2
Results of grafting freshly collected gonadal tissue that had never been frozen in the different donor/recipient combinations.

Donor/ Recipient	All recipients (*n*)	Allografted gonads		Age at termination (week)
Yellow Hungarian (YH)/White Hungarian (WH)	Female: 2 Male: 3	Female: 2 (100 %) Male: 2 (67 %)	average: 80 %	8
Yellow Hungarian (YH) /White Leghorn (WL)	Female: 7 Male: 3	Female: 3 (43 %) Male: 0	average: 30 %	8
Black Transylvanian Naked Neck (BTN)/ Novogen White (NW)	Female: 7 Male: 5	Female: 2 (28.5 %) Male: 1 (20 %)	average: 25 %	Female: 30 Male: 8
Partridge Color Hungarian (PCH)/White Leghorn (WL)	Female: 3 Male: 6	Female: 3 (100 %) Male: 3 (50 %)	average: 67 %	11

Table 3
Results of grafting frozen/thawed gonadal tissue in the different donor/recipient combinations.

Donor/Recipient	All recipients (*n*)	Allografted gonads		Age at termination (week)
Partridge Color Hungarian (PCH)/ Novogen White (NW)	Female: 5 Male: 7	Female: 5 (100 %) Male: 3 (43 %)	average: 72 %	8
Black Transylvanian Naked Neck (BTN)/ White Leghorn (WL)	Female: 5 Male: 3	Female: 2 (40 %) Male: 2 (67 %)	average: 50 %	Female: 21 Male: 8
Partridge Color Hungarian (PCH)/White Leghorn (WL)	Female: 6 Male: 6	Female: 3 (50 %) Male: 1 (17 %)	average: 33 %	Female: 23 Male: 8
Yellow Hungarian (YH)/White Leghorn (WL)	Female: 7 Male: 6	Female: 2 (28.5 %) Male: 2 (33 %)	average: 31 %	8
Speckled Transylvanian Naked Neck (STN)/ Novogen White (NW)	Female: 19 Male: 8	Female: 3 (16 %) Male: 3 (37.5 %)	average: 22 %	Female: 52 Male: 8
Speckled Hungarian (SH)/Novogen White (NW)	Female: 24 Male: 3	Female: 2 (8 %) Male: 1 (33 %)	average: 11 %	Female: 52 Male: 8

Tissues were sliced into 2–4 pieces. Tissues to be used for allografting without freezing were placed in Dulbecco's Modified Eagle's Medium (DMEM) at room temperature. The pieces of testicles and ovaries remained in the medium for 10–90 min before being transferred to the recipient birds (Liptoi et al., 2013). Gonadal tissues that were to be subsequently frozen were collected as entire testes or ovaries and placed in Dulbecco's Phosphate Buffered Saline (DPBS) and 20 % Fetal Bovine Serum (FBS) solution at 0 °C for no longer than 25 – 30 min.

2.3. Vitrification of donor organs

Three pieces of the prepared gonadal tissues were drawn into a human hand acupuncture needle (0.18 mm × 8 mm – Dongbang Acupuncture Inc., Korea). These needles were subsequently placed in two different vitrification solutions for 5 min each at room temperature using a slightly modified method of Wang et al. (2008). The content of the vitrification solutions was as follows: DPBS + 20 % FBS + 7.5 % Dimethyl Sulfoxide (DMSO) + 7.5 % ethylene glycol (EG) and DPBS + 20 %FBS + 15 % DMSO + 15 % EG + 0.5 M sucrose. After the equilibration period, the needles were immersed in liquid nitrogen and placed in cryovials of 1 ml volume (Labsystems Ltd., Hungary). The cryovials were closed with long forceps under liquid nitrogen, and were then placed in nitrogen tanks for long-term storage until tissue transfers were performed.

2.4. Thawing of the donor tissues

Immediately before allotransplantation, the needles containing the tissues were placed in three types of thawing solutions for 5 min each at 38.5 °C, using aseptic conditions (Wang et al., 2008). In the order of placement in solutions, the composition of the solutions was as follows: DPBS + 20 % FBS + 1 M sucrose; DPBS + 20 % FBS + 0.5 M sucrose; and DPBS + 20 % FBS + 0.25 M sucrose. Until allotransplantation, the tissues were stored in a solution of DPBS + 20 % FBS for no longer than 1 h at 0 °C. After thawing, donor tissues were sliced into 2–4 pieces with iris scissors.

2.5. Surgical transfer of gonadal tissues

Using the procedures described by Liptoi et al. (2013), anesthesia of recipients was induced using 0.1 mg xylazine (Narcoxyl 2) and 0.5 mg ketamine (CP-Ketamine 10 %) per chick, administered intramuscularly. During the period when allotransplantations were performed, anesthesia was maintained with isoflurane (Forane) administered using a mask. After the feathers had been removed from the abdominal area, the skin area was cleansed with 70 % ethanol. The chicks were subsequently placed in a recumbent position on a heating pad. A medial laparotomy approach was used with careful removal of the yolk sac occurring. In cases where only the allograft of donor gonads was evaluated, the recipient gonads were left intact. In contrast to this type of tissue transfer, when the recipients

were produced for progeny tests an ovariectomy was performed by ablating the cranial, middle and caudal parts of the ovary with a bipolar electrocautery device (Kentamed 1E) using a setting of 16 W. Because the ovary is located close to the primary blood vessels (aorta, vena cava), the ovarian ablation needs to occur with great care, especially the cranio caudal portion, so as to avoid excessive bleeding (Buda et al., 2019). In males, the technique used for gonadal tissue transfer was the same as in females, taking into consideration the location of the testes, which is also close to the aorta and the vena cava (Song and Silversides, 2006, 2007a, 2007b; Liu, 2013; Liptoi et al., 2013). The donor gonad was allografted as close as possible to its anatomic position of the gonad that was removed and was anchored by the parietal layer of the abdominal air sac. After the tissue allotransfer procedures were completed, the abdominal cavity was closed with two layers of polyglactin sutures (Safil 4.0). Chicks were treated with 0.05 mg dexamethasone IM postoperatively to prevent an acute immune reaction. There was oral administration daily of an immunosuppressant for 2 weeks and then once a week for an additional 6 weeks to individual birds at a dose of 4 mg/kg body weight mycophenolate mofetil (Song and Silversides, 2006, 2007a; Song et al., 2012; Liptoi et al., 2013).

2.5.1. Ablation of gonadal tissue without allografting

Kentamed 1E bipolar electrocautery was used with the setting being 15–30 Watts to ablate the ovarian tissue of chicks that were 1 day post-hatching (Table 5).

2.6. Housing of the birds

After the allotransplation of gonadal tissues, the chicks were placed in chicken-rearing blocks with zeolite litter being used until they were 5 weeks of age. Temperature and light were programmed according to age. From the fifth week the birds were transferred together to a pen with deep litter. All of the experimental birds were fed *ad libitum*, with starter diet until they were 16 weeks of age, and subsequently with pre-layer diet from 16 to 18 weeks and then with a layer diet after 18 weeks of age. The number of hours of lighting was increased gradually until there was 14 h of lighting in a 24 h period. The recipient hens, which were inseminated artificially for progeny tests, as well as the cockerels used for the insemination were housed individually in cages from the beginning of egg laying (24th week) while imposing a 14L:10D photoperiod. The collection of eggs for incubation lasted for 13 weeks, while in cases when donor-derived offspring were obtained (based on feather color markers) during that period, the egg collection was extended to 25 weeks.

2.7. Artificial insemination

Semen was collected using the dorso-abdominal massage method (Burrows and Quinn, 1937). The females were inseminated with fresh, undiluted semen. Insemination occurred twice weekly using 300 million spermatozoa per hen.

2.8. Histology

When there was allotransplantation of gonadal tissues that were not positioned in the proper anatomical location, histological examinations were conducted. The samples were placed in 35 % formaldehyde solution and then were embedded in paraffin. This was followed by preparing sections and staining the tissue samples with hematoxylin and eosin (Liptoi et al., 2013).

2.9. DNA analysis

Molecular genetic markers were used to confirm the donor origin of the ovarian tissues. Samples were collected from the ovary that was supposedly derived from the donor, and liver samples of the recipient were used for comparison. Furthermore, tissue samples (phalanges) were collected from those progeny 1 day after hatching that were thought to have been derived from the donor tissue that was allotransplanted based on their feather color pattern, and blood samples were collected from the recipient females and the males used for artificial insemination. Tissue samples were placed in nuclei lysis buffer (1 M Tris-HCl, 0.4 M NaCl, 1 mM EDTA, pH 8.2), and blood samples were placed in Na citrate (1:1) at –20 °C until DNA isolation. The DNA samples were extracted using the traditional salting-out method (Miller et al., 1988) modified for chickens (Bodzsar et al., 2009). The DNA concentration was determined using a Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific), and the samples were equilibrated to concentrations of 5 ng/μL. The microsatellite markers used in the present study (*ADL0278, MCW0037, MCW0034, MCW0123, MCW0067* and *MCW0080*) were selected from the Aviandiv Project, 2003 (https://aviandiv.fli.de/primer_table.html). Locus *MCW0080* (forward: 5'-GAAATGGTACAGTGCAGTTGG-3'; reverse: 5'-CCGTGCATTCTTAATTGACAG-3') was excluded from the list recommended by the FAO, however, it was included in the present study because these six microsatellites had been used previously in multiplex PCR reactions (Kyratec trinity supercycler), which means that the genotyping was completed successfully with more markers at the same time. The fragment analysis was performed using capillary gel electrophoresis utilizing a Beckman Coulter automatic DNA sequencer (GenomeLab™ GeXP Genetic Analysis System) according to the manufacturer's instructions. Genotyping data were analyzed using the GenomeLab GeXP System fragment analysis software (Beckman Coulter) utilizing a 400 bp size standard for allele size identification.

3. Results

By allotransplanting of recently collected tissues that had never been frozen, there was determination of whether the

allotransplanted tissues had the capacity to serve as an allograft and develop normally when there were specific donor/recipient genetic combinations evaluated. The results for the genetic pairing combinations are provided in the information included in Table 2. In the YH/WH donor/recipient combination, the successful allografting rate of donor gonads was as great as 80 %. The results from tissue evaluations at 8 weeks post-hatching and the histological examinations indicated that the allotransplanted gonadal tissue had the capacity for gametogenesis. Based on these results and also considering the genetic differences, there was initiation of the study evaluating the YH/WL allograft combinations with the gonadal tissue allotransplantations. The use of this donor/recipient combination resulted in a 30 % allografting success rate when there was gonadal allotransplantation using these breed combinations. Because the NW is a WL-based egg layer hybrid, this genotype was used as a recipient for the Black Transylvanian Naked Neck (BTN) tissue allotransplantations. The success rate of gonadal tissue allografting in the recipient birds using these breed combinations was 25 %. For the PCH/WL tissue allotransplant combination, the success rate for gonadal tissue allografting was 67 %. With regard to differences between the sexes, there was always a greater success rate for ovarian than with testicular allografting.

Data are included in Table 3 for the results of allotransplanting frozen/thawed gonadal tissue in various combinations based on genetic differences among breeds. For the following donor/recipient combinations the successful gonadal tissue allografting rates were: 72 %, PCH/NW; 50 %, BTN/WL; 31 %, YH/WL; 22 %, Specked Transylvanian Naked Neck (STN)/NW; and 11 %, Speckled Hungarian (SH)/NW. The data validating the tissue origin of donor gonads was verified using microsatellite analysis. As for the sex-related differences in frozen/thawed tissue allotransplantations, there was a slightly greater proportion of the testicular tissues for which there was successful allografting than for ovarian tissues.

Recipient hens of the STN/WL allotransplantation were developed for progeny testing ($n = 19$). A total of 58 % of these hens produced eggs (11/19). It was impossible to evaluate the progeny from 15 % (3/19) of the hens because, unfortunately, there were ovulations into a demarcated serous membrane capsule. Overall, 9.1 % (1/11) of the recipients produced donor-derived offspring. Of the eggs ($n = 87$) of that hen, 58.6 % (51/87) hatched of which 21.4 % (11/51) of the eggs were from the donor-derived tissues based on feather color pattern of the offspring. The eggs of the other offspring were derived from the ovarian tissues of the recipient.

Based on data obtained using microsatellite analysis, some alleles were detected in every individual, which must come from only the male and the donor-derived ovarian tissue, because the tissues of the recipient female does not have that allele. These allele combinations provide evidence that allow for discounting the recipient animal as the source of these alleles in the progeny. The different alleles detected in the recipient blood and ovarian tissue provide convincing proof for the donor derivation of the ovary (Table 4).

The results of ovariectomy by electrocautery (without allotransplantation) indicate that it was possible to select appropriate electrocautery settings that enabled the removal of the entire organ without any damage to surrounding tissues. A demarcated serous membrane capsule, however, also developed as a result of conducting these procedures in some birds (Table 5).

4. Discussion

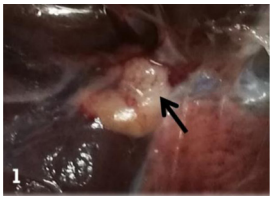
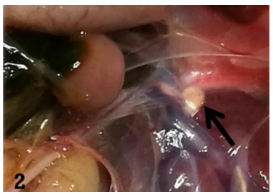
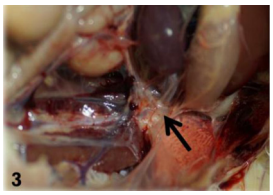
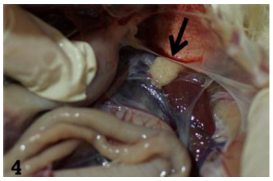
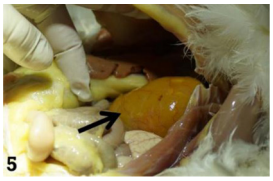
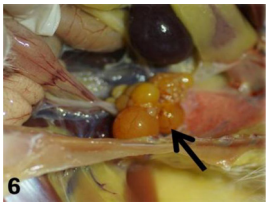
The surgical technique of gonadal allotransplantation at 1 day post-hatching has been utilized successfully in several avian species and different breeds of birds as well (Song and Silversides, 2006, 2008a; Song et al., 2012; Liu et al., 2013). The efficiency in which

Table 4
Allele sizes (base pair) of the males used for artificial insemination, of the recipient hen's blood and donor-derived ovarian tissue, and of the progeny based on microsatellite loci.

Microsatellite loci	ADL0278		MCW0037		MCW0034		MCW0123		MCW0067		MCW0080	
Male 1	130	130	171	174	250	250	107	109	194	196	300	300
Male 2	131	131	171	175	242	242	99	109	194	200	300	300
Male 3	130	130	171	173	244	254	105	109	194	200	298	298
Male 4	130	140	171	171	242	254	107	107	196	200	298	300
Recipient female blood	130	137	173	173	242	242	105	105	194	194	296	296
Donor-derived ovarian tissue	130	140	173	175	242	254	103	107	194	200	296	300
Progeny 1	130	130	171	175	250	254	103	109	194	200	296	300
Progeny 2	130	140	173	174	242	242	103	109	194	194	300	300
Progeny 3	130	140	171	173	250	254	107	107	194	194	300	300
Progeny 4	130	130	173	174	242	254	99	107	200	200	300	300
Progeny 5	130	140	171	175	242	242	103	109	194	194	298	300
Progeny 6	130	130	173	175	242	242	103	109	200	200	300	300
Progeny 7	130	130	173	175	242	254	103	105	194	196	296	298
Progeny 8	130	140	173	174	250	254	103	107	194	196	300	300
Progeny 9	130	130	171	173	250	254	107	107	194	194	300	300
Progeny 10	130	140	171	175	242	242	107	109	194	200	300	300
Progeny 11	130	130	174	175	242	242	103	109	200	200	296	300

Alleles marked with gray color indicate there was exclusion from being included as a recipient in the progeny.

Table 5
Results of ovariectomy when using cauterization (marked with black arrows on the photos) (n = 6).

Day-old females	Age of termination (week)	Cauterization technique with different electric power (Watt)	Autopsy result	Photo of the result
1st	9	Several pieces 30 W, then 15 W	Small piece of ovary left	
2nd	9	15 W, then 20 W	No ovary left	
3rd	18	20 W cranio caudal direction	No ovary left	
4th	18	20 W in the middle part first, then cranially and caudally	About three-fourths of ovary left	
5th	18	18 W cranio caudal direction	Demarcated serous capsule, including ovary, ovulated follicles. The tuba is divided	
6th	18	18 W caudocranial direction	Entire ovary left	

there is successful donor gonadal tissue allografting, however, has not been ascertained, especially in domestic chicken breeds. While there are genotypes in which gonadal tissues can be combined successfully, in other cases the ratio of functioning grafted donor tissues is small or zero. Pre- and postoperative treatments had no effect on the success of allografting (Liptoi et al., 2013). These findings are unlikely to be only due to underlying immunological effects. Allografting, however, can be successful without any immunosuppressant treatment (Song and Silversides, 2007a, 2008b; Liptoi et al., 2013). Gonads are immune-privileged organs for which the probability of rejection is less than with many other tissues (Hedger, 2007). The reason for lack of successful allografting may be the result of developmental biological incompatibility. There is a difference in the sexual maturation of different genotypes, which also may have affects during the early stages of gonadal development. Another possible reason for the lack of successful tissue allografting relates to immunological tissue incompatibilities, which is not related to the rejection of the tissue allograft but affects the functional integration of the allografted gonadal tissue (for example formation of blood supply). Results from previous studies (Liptoi et al., 2013) as well as those from the present study, indicate that freshly collected tissues that have never been frozen can be allotransplanted with a 70%–80% of functional allografts if the recipient genotype is genetically compatible.

Liu et al. (2010, 2013) produced donor-derived offspring by allotransplanting frozen/thawed ovarian tissues in Japanese quail,

but in domestic chickens there have not been any reports of these allotransplants resulting in production of donor-derived offspring. Lessard et al. (2017) grafted 15 ovaries and seven testes to recipient-line animals. One of the ovaries was successfully allografted, however, the allografted tissues did not have the capacity for gametogenesis. There, however, were 50 % of the donor testes that were transferred to recipient line animals that had functional gametogenesis. Results of the present study indicate that when there is use genetically compatible recipients the successful allografting of frozen/thawed tissues can be nearly as efficient as that reported for allotransplantation of recently collected gonadal tissues that have never been frozen (72 %). Furthermore, donor-derived progeny can be obtained as a result of gametogenesis in the allografted ovarian tissue and there be subsequent production of eggs with the genetic lineage of the allografted ovarian tissue. The slightly modified thawing protocol of Wang et al. (2008) could also be an important technical aspect in the successful gonadal tissue allotransplantations. In addition, the use of this protocol facilitates the maintenance of the integrity of tissues. This results because the solutions are used in larger amounts so that the relatively large needle utilized for allograft transfers does not result in a cooling of the allotransplanted tissues to the extent that may occur with other procedures and allows for more rapid allotransplanted tissue warming during the thawing process.

With regard to the sex-related differences in the successful allografting of tissues, in earlier studies when there was allotransplantation of tissues that were recently collected from donor animals and that had never been frozen, there were no such differences in the success rate for allografts in the recipient animals (Liptoi et al., 2013), however, based on the results in the current study there was a greater success rate for allografting when there was allotransplantation of ovarian tissues (at a rate of 100 % in many instances) than testes regardless of genotype. One of the possible reasons for this finding is that ovarian tissues that have never been frozen have a greater capacity for vascular anastomosis than testes which having a thicker surrounding capsule. When there was grafting of frozen gonadal tissues, there was a slightly greater success rate for allografting of testicular tissue in the present study. It, however, is possible that there is a greater tolerance with testicular tissues for cryopreservation. Another possible reason could be related to the results of the present study that among females that were produced for progeny tests there was a demarcated serous membrane capsule that developed in some of the hens. This capsule prevented the ova from reaching the oviduct, therefore, there was not a subsequent oviposition. The donor origin of the ovary in these birds, therefore, could not be verified. Furthermore, there can be a difference in the allografting success rate when allotransplanting tissues and the capacity for gonadal tissues to remain viable after use of freezing/thawing procedures between genotypes and individuals as well.

Ovariectomy in the recipients can be performed with a forceps and iris scissors; however, it requires extremely precise manual dexterity and great practice, because excessive bleeding can be induced due to the location of the ovary (Song and Silversides, 2006; Buda et al., 2019). Electrocautery seems to be a more suitable method for performing this gonadal ablation technique. It should be noted, however, that with both techniques it is difficult to avoid having a small piece of recipient ovary retained along with the donor allografted tissues, and therefore, the offspring can be derived from both the ovarian tissues of donor and recipient when there is not total removal of the recipient ovarian tissues. There have also been some reports on the development of the previously described serous membrane capsule in the abdominal cavity of the hens when ovariectomy procedures have been performed (Kosenko, 2007; Song and Silversides, 2007a), but in these previous studies only scissors were used for removing the ovary. The question has arisen whether the surgical technique or the donor tissue allotransplantation was the reason for development of this serous membrane capsule. When there was no tissue allotransplantation but functionality of ovarian tissues was ablated by cauterizing the gonadal tissue, it appeared as though the capsule was formed because of the scarring and the formation of this capsule occurred when there was no donor tissue allotransplantation. By optimizing cauterization, this problem may be avoided in the future. The correct wattage utilized for ablations can vary with the different devices; thus, further evaluations are recommended to determine if there is a wattage that could be used to avoid the development of these serous membrane capsules.

Selection based on the genetic differences of genetically compatible pairs seems to be an effective method for producing birds with appropriate donor/recipient combinations for having successful allografting of gonadal tissues. More precise determination of the genetic/physiological background requires further investigations, which can facilitate the selection of recipient birds for donor genotypes when performing gonadal allotransplantations. With use of this method, there can not only be gonadal tissues of females of the indigenous or endangered chicken breeds preserved, but there can also be preservation of the genetic pool of commercial lines that at the present time are not needed for genetic development by the breeding companies but that may be useful in the future as a genetic resource when needs of these companies and/or the poultry industry are different. With the use of these allografting techniques, the whole donor genotype can be rapidly reproduced with the production of an F1 generation if there is a need to do so.

CRediT authorship contribution statement

Krisztina Liptoi: Conceptualization, Methodology, Supervision, Writing - original draft, Investigation, Validation, Funding acquisition. **Kitti Buda:** Writing - original draft, Investigation, Validation, Visualization. **Emese Rohn:** Investigation. **Arpad Drobnyak:** Investigation. **Erika Edvine Meleg:** Investigation. **Nora Palinkas-Bodzsar:** Writing - original draft, Investigation, Methodology. **Barbara Vegi:** Investigation, Methodology, Validation. **Judit Barna:** Supervision, Writing - review & editing, Methodology.

Declaration of Competing Interest

The authors have no conflict of interest to declare.

Acknowledgement

This research was supported by Horizon 2020 IMAGE n°677353 and GÉNNET_21 - VEKOP-2.3.2-16-2016-00012. Special thanks to András Hidas for his help in the microsatellite analysis.

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