poultry

MDPI

*Article*

# The Male Primary Sex Ratio Bias in Goose Eggs Early in the Laying Season: A Pilot Study

**Valeriy G. Narushin [1], Michael N. Romanov [2,3,4,*], Darren K. Griffin [2,3], Sabine Klein [5], Attila Salamon [6], Sara Copeland [7], Cormac O'Shea [8] and John P. Kent [7]**

[1] Independent Researcher, Soborny 145-B, 69035 Zaporizhya, Ukraine; val@vitamarket.com.ua
[2] School of Natural Sciences, University of Kent, Canterbury CT2 7NJ, Kent, UK; d.k.griffin@kent.ac.uk
[3] Animal Genomics and Bioresource Research Unit (AGB Research Unit), Faculty of Science, Kasetsart University, Chatuchak, Bangkok 10900, Thailand
[4] L. K. Ernst Federal Research Center for Animal Husbandry, Dubrovitsy, Podolsk 142132, Moscow Oblast, Russia
[5] Friedrich-Loeffler-Institut, Mariensee, Institute of Farm Animal Genetics, 31535 Neustadt, Germany; sabine.klein@fli.de
[6] Department of Ethology, ELTE Eötvös Loránd University, H-1117 Budapest, Hungary; dr.attila.salamon@gmail.com
[7] Ballyrichard House, Y14 FC95 Arklow, Co. Wicklow, Ireland; scc096@mail.usask.ca (S.C.); j.p.kent.82@cantab.net (J.P.K.)
[8] Department of Bioveterinary and Microbial Sciences, Faculty of Science and Health, Technological University of the Shannon, Midlands Midwest, N37 HD68 Athlone, Co. Westmeath, Ireland; cormac.oshea@tus.ie
* Correspondence: m.romanov@kent.ac.uk

## Abstract

In bird eggs, the theoretical expectation of a primary sex ratio (at conception) of 50:50 males/females often fails to materialize. Using PCR technology for sex verification in this pilot study, we evaluated the primary sex ratio of 128 fertilized domestic goose eggs (*Anser anser*) early in the laying season. Over 24 consecutive days of egg collection, 37% more males were found (58% males vs. 42% females). This male-biased trend gradually declined over the period, but an excess of males was still observed. Among the factors for predicting the male sex ratio bias in a particular goose was the egg weight, i.e., heavier eggs tended towards a male phenotype. The embryo sex of the first egg laid and the egg weight change dynamics over the laying period were also noted. The correlation between actual and predicted data was calculated, taking into account three parameters, and found to be 0.724. To explain the effect of an implicit random/non-random process more adequately, we introduced the concept of *biased randomness*. As well as being of academic interest, research on sex ratio bias is crucial for goose breeding/reproduction programs and important as a step towards understanding the physiological mechanisms that underly sex ratio bias in these animals.

**Keywords:** *Anser anser*; goose eggs; embryos; sex ratio bias; egg weight change dynamics; random series generation and estimation

Academic Editors: Marcin Wojciech Lis and Barbara Tzschentke

Received: 22 September 2025
Revised: 3 November 2025
Accepted: 20 November 2025
Published: 1 December 2025

**Citation:** Narushin, V.G.; Romanov, M.N.; Griffin, D.K.; Klein, S.; Salamon, A.; Copeland, S.; O'Shea, C.; Kent, J.P. The Male Primary Sex Ratio Bias in Goose Eggs Early in the Laying Season: A Pilot Study. *Poultry* **2025**, *4*, 59. https://doi.org/10.3390/poultry4040059

## 1. Introduction

According to Hardy [1], the growing understanding of sex ratio variation across vertebrate animals, including livestock species, is one of the more significant developments in evolutionary ecology. Moreover, there is still considerable disagreement and ambiguity surrounding adaptive reasons for these variations. Inferring process from pattern can be challenging, in part because competing explanations can often lead to contradictory conclusions.

Male and female avian embryos should theoretically be expected to be produced in equal proportions during fertilization, since the avian ovary generates Z- and W-bearing oocytes in equal quantities. This conforms to the notion of the primary sex ratio in animals that have heteromorphic sex chromosomes [2]. In practice, however, the situation is somewhat different in that, amongst hatched chicks, a secondary sex ratio different from 1:1 is observed. That is, some embryos die during the incubation process and the subsequent secondary sex ratio observed varies depending on the bird species, breed, genetic features, age of laying hens, ratio of females to males in a flock, etc. [2–8]. As such, data are difficult to collect given the number of variables involved. Whether differential mortality completely explains biases in hatching sex ratios (i.e., are there other contributing factors?) is the subject of some debate in the literature; however, laying hens can directly influence the primary sex of their offspring. This adaptive feature was addressed in birds [1,9], and equivalent processes have been observed in mammals [10–15], among other groups.

At a causal level, Pike and Petrie [16] and Alonso-Alvarez [17] suggest that regulation of offspring sex can be influenced by a change in the bird's hormonal environment. An altered sex ratio during the reproductive period has been indicated in chicken of different genotypes [18,19]. The mechanisms for such adaptive changes can involve anatomical, physiological, social, and genetic factors inherent in an individual, as well as external environmental parameters [20–24]. To date, however, the causal mechanisms have not yet been fully established.

When Narushin et al. [25] assessed the possible causes of sex ratio bias in ducks of a commercial flock over seven years (2014–2020), key factors were identified that, perhaps to some degree, influence, or are associated with, the shift in the embryo sex in one direction or the other. These include (1) periods of duck egg lay, i.e., a growth or decline in the number of eggs laid, and (2) weather conditions. For the latter, air temperature and wind strength were the most significant, but these may be features of a more general seasonal trend that could be associated with other yet unidentified causal mechanisms. Since in that study, sex bias was measured as a flock average, it was not possible to reveal effects at the level of the individual hen. That is, in a flock, different birds may be at different phases of fecundity: for some, egg-laying growth might have already begun, whereas others may still be in the deep recession or, perhaps, in the molt period.

In this investigation, we examined a flock of domesticated individual geese of the root species *Anser anser* (sometimes referred to as *A. a. domesticus*; for brevity, we here refer to these as *A. anser*). Notably, we took a different approach to the study of geese as they are among a few domesticated avian species characterized by seasonal egg laying. We started the experiment at the very beginning of their egg production period and, thus, all females were in the growth cycle phase. According to Narushin et al. [25], this is especially critical in influencing the embryo sex, while the weather factors are common to all individuals. Trivers and Willard [26] hypothesized that possible sex bias (especially in seasonal breeders) is influenced by parental condition. While parents in poorer health should have daughters, parents in better health should generate sons. Toward the end of the laying period, the female's condition deteriorates, while the male's condition remains virtually unchanged. This imbalance leads to the expectation that a healthy male father has a much greater impact on egg production than an unhealthy female parent. This factor is of no small importance and significantly influences the sex ratio shift at the end of the laying period. Since we were primarily interested in identifying embryo sex directly from egg parameters associated with a specific bird, without considering the characteristics of the parents, this fact further confirmed the usefulness of conducting research at the beginning of laying, i.e., during the production growth phase, when the parents are in equal physiological conditions.

Thus, the following reasons can be given in favor of studying the primary sex ratio of goose embryos at the beginning of the productive period of egg-laying by their parents:

- Both parents are in approximately the same physiological state, and the female's basic resources are not yet depleted;
- Weather factors do not affect the possible adjustment (shift) of the sex ratio;
- As a consequence, the first eggs of the laying season better reflect the genetic and physiological standard, which is confirmed by numerous studies conducted on both wild and domestic goose species [27–32].

In this study, we therefore considered only the primary sex ratio determined by PCR analysis. The purpose of this pilot study was hence to test the hypothesis that there are significant differences in sex ratio at the primary level in geese early in the laying season and, if so, to ask the question, in what direction is the change?

## 2. Materials and Methods

### *2.1. Study Flock*

This pilot study was performed in as naturalistic an environment as possible in a domestic waterfowl farm in Ireland, working with a flock of Legarth geese from Ballyrichard Farm (Arklow, Ireland; 52°50′5″ N, 6°7′49″ W). The appropriate flock management and housing criteria were adhered to as described elsewhere [27–29]. Twenty female geese were selected based on finding them laying in a nest that they would continue to use for their clutch of eggs. They were labeled using spray paint with individual markings and assigned individual identities with letters from A to V. It should be noted that not all letters of the alphabet from this series were used and, therefore, the absence of some of them further in the text does not mean their accidental omission or departure during the experiment process.

### *2.2. Egg Handling and Molecular Sexing*

During the observed laying period (from 10 February to 5 March 2021), a total of 135 goose eggs were obtained. The mean number of eggs per goose was 6.7 ± 1.2, with a median of 6 and a range of 5 to 9 eggs between geese. Each egg was weighed ($W$) using an electronic scale to the nearest 0.01 g. The eggs were collected and stored on farm at 6 °C to complete the whole batch for the experiment and then transported in isolated egg transportation boxes for molecular sex identification analysis. The fertility state was visually inspected at the blastoderm stage without incubation. The germinal disks of all eggs were separated for the further polymerase chain reaction (PCR) analysis of the sex-specific *CHD1* gene sequences. The latter are highly conserved in most birds and can be used as universal molecular sexing markers as described elsewhere [33–37], with some modification [38].

Processing of the germinal disk samples for DNA extraction and sex-specific PCR was performed for all eggs of the individual goose in one batch. Germinal disks were separated from the yolk and frozen, and DNA extraction was performed using alkaline lysis. The samples were dissolved in 280 μL lysis buffer (100 mM Tris-HCL, pH 8.0, 100 mM NaCl, 100 mM EDTA, 1% SDS and 10 mg/mL Proteinase K for 3 h at 56 °C). After precipitation of proteins in saturated NaCl, the DNA was washed in ethanol several times, dried, dissolved in TE buffer, and stored at −20 °C. For PCR (using GoTaq® G2 Hot Start Taq Polymerase, M7405, Promega, Madison, WI, USA; and *CHD1*-specific PCR primers as listed in Table 1), the total amount of 25 ng DNA was applied as a template and 35 cycles were run at 57 °C annealing temperature. After PCR amplification, the Z- and W-specific PCR products were evaluated via 2% agarose gel electrophoresis. Molecular sexing was achieved from 128 out of 135 eggs; non-informative eggs were caused by technical and preparation losses.

**Table 1.** Forward (F1, F2) and reverse (R) primer sequences used for molecular sexing.

| Primer | Sex-Specific PCR Primer Sequences | Amplicon |
|---|---|---|
| F1 | 5′-GTTACTGATTCGTCTACGAGA-′3 | 447 bp (*CHD-W*)<br>593 bp (*CHD-Z*) |
| F2 * | 5′-GCTACTGATTCGTCTGCGAGA-′3 | |
| R | 5′-ATTGAAATGATCCAGTGCTTG-′3 | |

* Primer F2 was modified according to reference sequences for chicken Z chromosome. All primers matched the goose Z and W chromosome sequences, implying that they can be used for molecular sexing in geese.

### *2.3. Statistical Analyses*

For mathematical representation, all eggs in which a genetically male embryo was found were assigned a quantitative index of 1, and the group of eggs with genetically female embryos was, respectively, assigned a quantitative index of 2.

Since in our studies there was no technical ability to evaluate absolutely all eggs laid during the entire productive period, we limited ourselves to only the first month of egg laying in the given goose population. Considering that over the entire period, i.e., from February to June, the females would perhaps have laid approximately three times more eggs [27,28], we needed to assess the representativeness of this experimental sample. For this purpose, we implemented the calculation formula for minimum sample size from Cochran [39] and the appropriate assumption on the margin of error ($E$). Cochran [39] left the choice of the acceptable value of $E_{\max}$ to the discretion of researchers, however, recommending its value at the level of 5% (0.05). Thus, our aim was reduced to calculating the $E$ value and comparing it with the permissible value of $E_{\max}$. If the calculated $E$ value does not exceed $E_{\max}$, i.e., 0.05, the sample can be considered representative. In this case, the Cochran's calculation formula for minimum sample size can be converted to the following:

$$E = \frac{(N - n)\sigma_y^2}{(N - 1)n}, \tag{1}$$

where $N$ is the total number of eggs that 20 females could lay during the entire laying cycle; $n$ is the number of eggs in the studied experimental sample; and $\sigma_y$ is the standard deviation of the parameter under study, which in our case conforms to the presence of a male or female embryo in the given egg. For the studied sample of 135 eggs, it can be assumed that over a full laying season, the 20 females could lay around three times more eggs, i.e., approximately 405 eggs. In this initial experimental sample, seven eggs were not evaluated.

The STATISTICA 5.5 software (StatSoft, Inc./TIBCO, Palo Alto, CA, USA) and Microsoft Excel computational tools were employed to process the numerical data obtained and assess the statistical significances using Student's $t$-test and other relevant tests, such as Pearson's $\chi^2$ goodness-of-fit test, Fisher's test, G-test, and the Wald–Wolfowitz runs test.

## 3. Results

### *3.1. Sample Representativeness*

The numerical series obtained in this pilot study included 74 "ones" (males) and 54 "twos" (females), reflecting the value of the function ($y$) for the presence of a male or female embryo in a goose egg.

For this series ($y$), the standard deviation ($\sigma_y$) was 0.50, which gave us the opportunity, using Equation (1), to calculate the $E$ value, which was 0.003. The obtained value of $E$

was almost 20 times less than $E_{\max} = 0.05$, indicating the representativeness of the egg sample studied.

### *3.2. Cumulative Results for the Study Flock*

The full data on eggs produced by each goose are presented in Supplementary Table S1. This includes the number of eggs laid by each female during the observed period, their *W* in g, and the ratio of male embryos (M) to female embryos (F). The respective graphic visualization in Supplementary Table S1 allowed us to track the trend of changes in these indicators.

Due to some technical failures in this pilot study, it was not possible to measure the *W* value in one egg of goose A and identify the embryo sex in one egg each of geese A, F, P, O, and Q and in two eggs of goose K. Therefore, in the graphic images of Supplementary Table S1, there may be discrepancies between the number of points corresponding to egg weight and those showing the sex of the embryo.

Analysis of the data produced (Supplementary Table S1) did not enable us to identify a general tendency for individual changes in the values of *W* and M:F across all geese. In some individuals, an elevation in *W* over 24 days of the assessed period was accompanied by a shift in the sex ratio towards females, while in the others there was, on the contrary, a male bias. Sometimes, the M:F trend line remained unchanged. A similar situation was observed in the case of a reduction in the *W* value during the first month of lay. In this regard, we decided to carry out a general assessment for the entire experimental sample of both females and the eggs they laid. Figure 1 provides an insight into the individual distribution of the data obtained regarding the mean *W* values from each goose (Figure 1a) and the sex ratio of males to females (Figure 1b).

Graphic visualization in Figure 2 helps us to assess the possible relationship between mean *W* and M:F. Despite a large scatter of parameters (the range of M:F showed the variations from 0.5 to 5), which reduced the accuracy of the trend line approximation (Figure 2), it was evident that the general trend of sex ratio changed from a clear predominance of males in eggs of lower *W* to a reduction in their component in heavier eggs. At the same time, when we used only the trend line as an information component, it was noticeable that, both for lighter and heavier eggs, the M:F ratio was above 1. In other words, despite the *W* value, the number of males in eggs always exceeded the number of females, although this tendency was significantly reduced in eggs of greater *W*. However, the correlation between mean *W* and M:F values was low ($R = -0.190$) and insignificant.

We also estimated possible differences in the *W* values of all the eggs that produced embryos of a different sex (M = 1 and F = 2). The results of the *t*-test showed an insignificant difference. The only thing that could be stated unequivocally was that the lightest egg contained a female embryo, while the heaviest one contained a male embryo (Figure 3).

It is not possible to assert definitively that there is an absence of a relationship between M:F and *W*, despite the insignificant results. This is due to the insufficient data to reject the null hypothesis. Nevertheless, the trends obtained here could be especially useful for conducting and analyzing further experiments.

We also attempted to track the general trend in the degree of excess of one sex over the other during the assessed period, along with the trend in *W* (Supplementary Figure S1). Both trend lines demonstrated a weak but stable trend of variability in the M:F and *W* values.

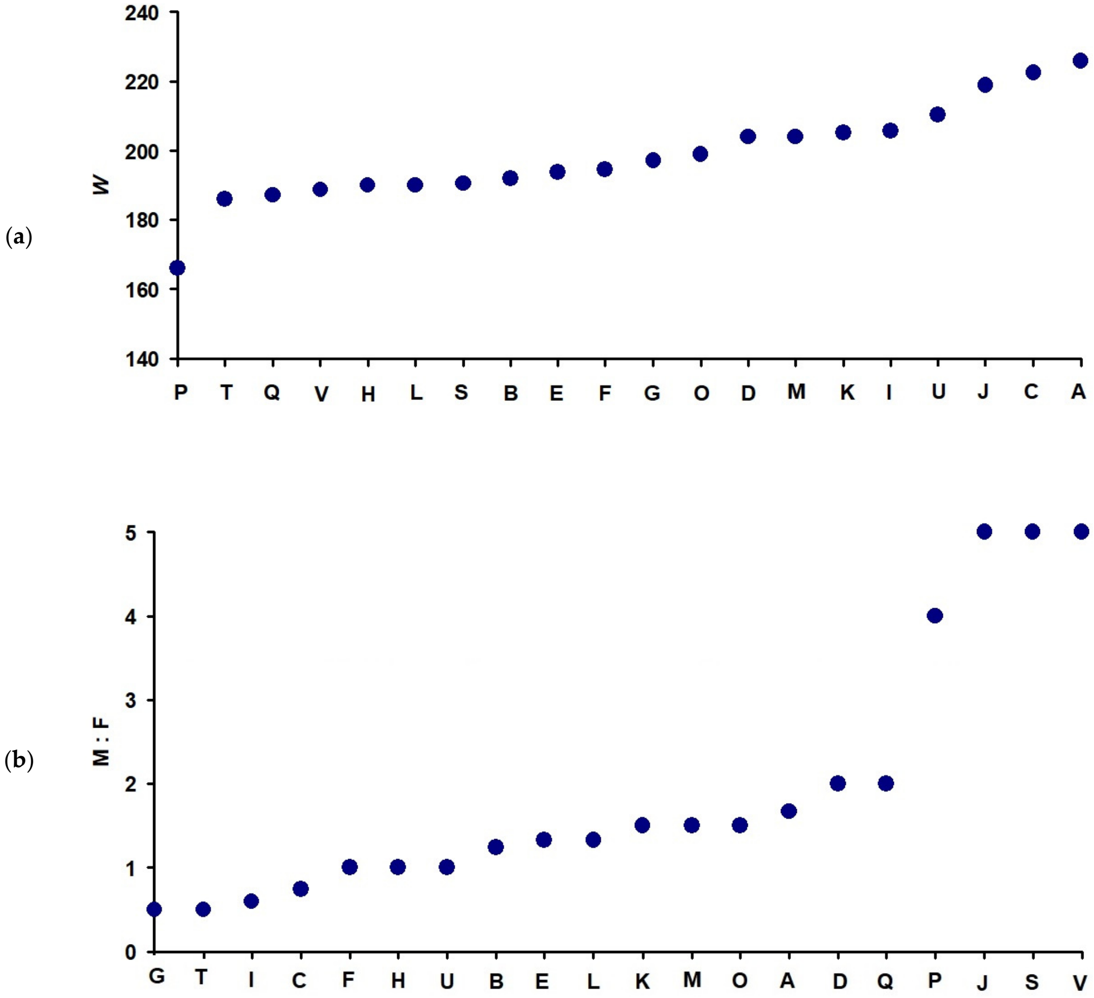

**Figure 1.** Mean data distribution across twenty females (A to V): (**a**) egg weight differences between the laying geese; (**b**) male-to-female ratios between the laying geese. The M:F ratio was calculated as the ratio of the number of eggs containing male embryos to the number of eggs containing female embryos.

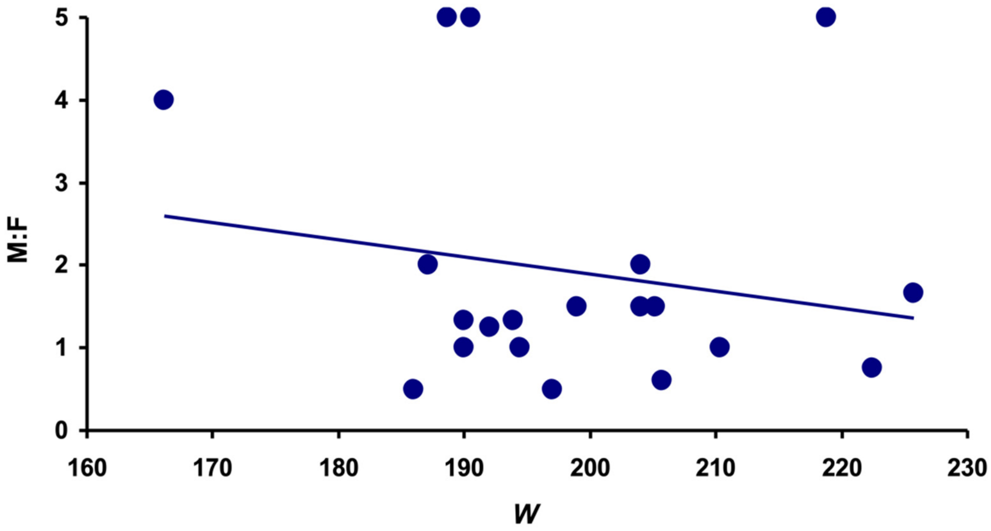

**Figure 2.** Graphical dependence of the mean egg weight ($W$) from each goose and the sex ratio of their embryos (M:F).

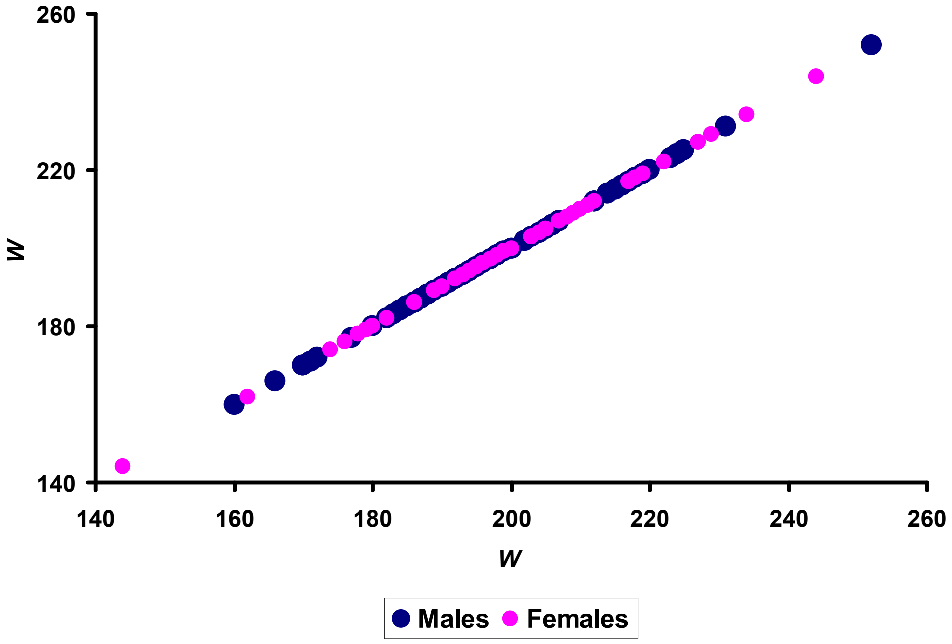

**Figure 3.** Visualization of egg weight (*W*) data relative to male (blue dots) and female (purple dots) embryos.

### 3.3. *Embryos in Heavy and Light Eggs*

As there is some relationship between sex ratio and *W* (Figure 2), we hypothesized that it could be tighter if we divided eggs into two groups using the linear algebraic group approach [40]. Applying this analytical approach, we sought to establish how smoothly the trend of decreasing M:F value with increasing *W* is traced. A large range of the parameters' values recorded during the analysis of this relationship (Figure 2) impugns the uniformity of this trend. The first group, which included eggs from 9 geese, was conventionally named "heavy eggs,", i.e., their *W* was above the mean value, whereas the second group (respectively, comprising eggs obtained from the remaining 11 geese) included "light eggs", with their *W* being correspondingly lower. Herewith, we considered the mean *W* values obtained from each goose during the assessed period. Subsequently, the resulting relationship between *W* and M:F was visualized in Figure 4.

The inferred trend lines enabled us to suggest that an increase in *W* of lighter eggs was associated with an excess of females, which is consistent with other studies [26]. However, the higher *W* values of the heavy eggs group gave a bias towards male embryos.

We also attempted to detect some distinctiveness of the changes in *W* and M:F when broken down at the individual level. The respective results for each goose are presented in Supplementary Figure S2.

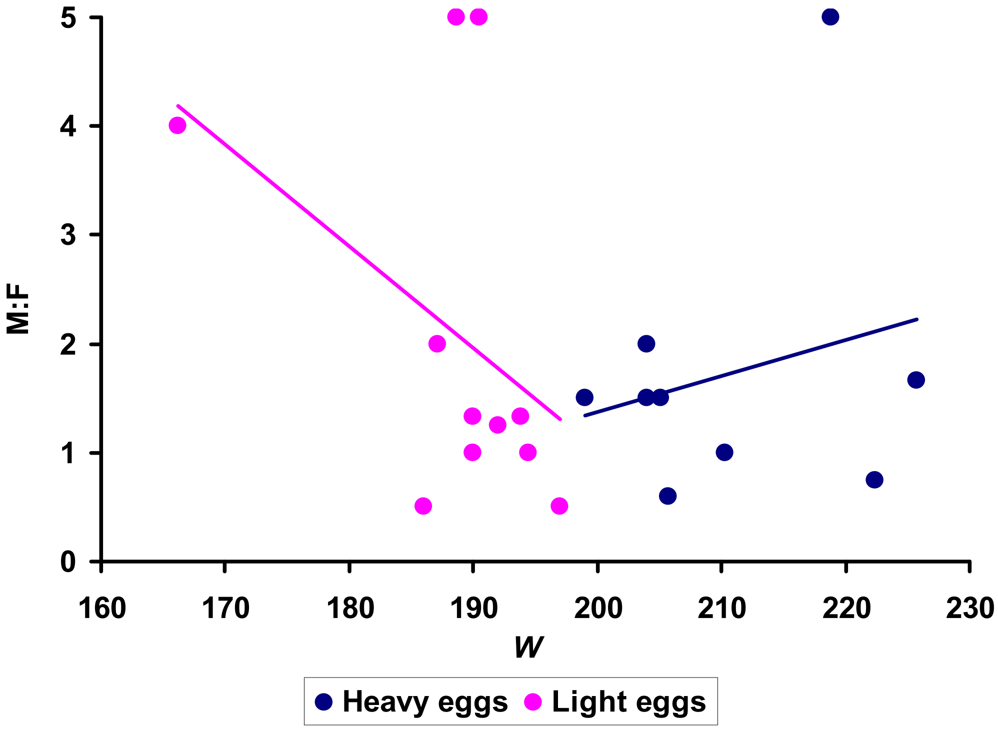

**Figure 4.** Graphical relationship of the weight (*W*) of heavy eggs (blue dots and line) and light eggs (purple dots and line) with the sex ratio of their embryos (M:F).

### *3.4. Embryo Sex in the First Egg*

When considering the first egg among the 20 females, 16 geese had a male embryo in their first egg, i.e., demonstrating a strong male primacy effect. However, the first eggs of the geese we labeled as K, O, and Q were not sexed due to a technical issue, and therefore, in our analysis we considered the sex of the embryo of the second egg she laid.

Considering the process of shifting the embryo sex during lay (Supplementary Figure S3), we conclude that most geese (13 of 16, i.e., 81.25%) that laid their first egg with a male embryo, gradually shifted to producing females by the end of the assessed period (Supplementary Figure S3a).

For convenience, the linear regressions calculated for each trend line of Supplementary Figure S3 are presented in Supplementary Table S2.

For each of the two groups considered, we tried to establish a possible relationship between the M:F ratio and *W*; however, the trend turned out to be identical. In both cases, with a rise in the *W* value, a lowering in the excess of males over females was observed (Figure 5).

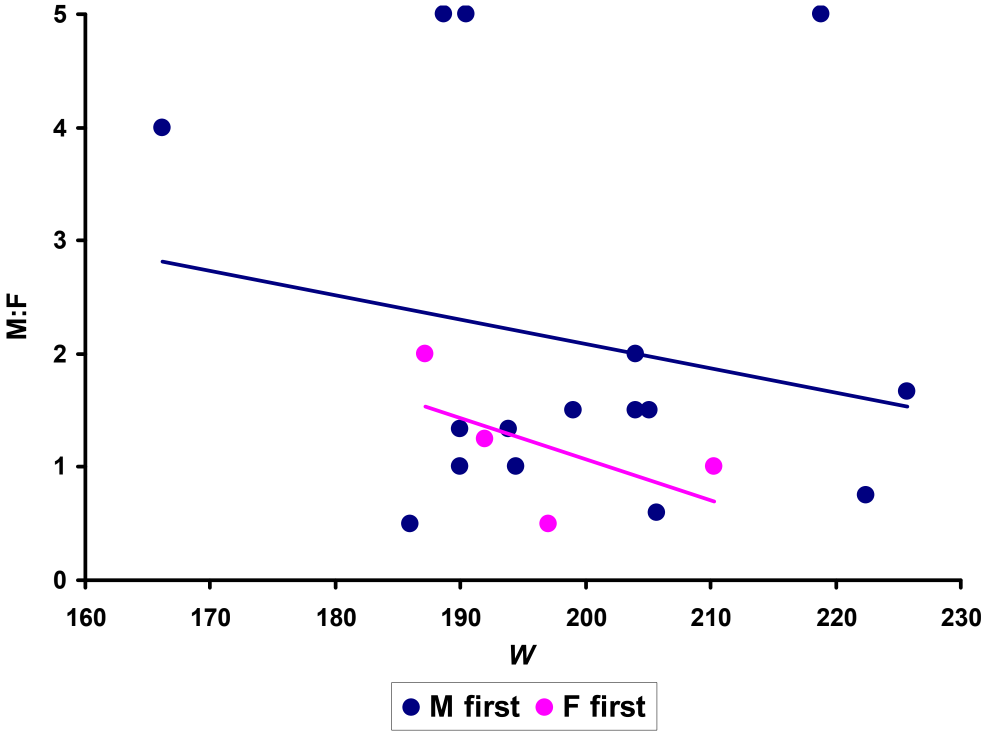

**Figure 5.** Relationship between the sex ratio of goose embryos (M:F) and egg weight (*W*) depending on the presence of a male or female embryo in the first egg laid.

### *3.5. Dynamics of Changes in W During the Egg-Laying Period*

If we consider the graphs of individual changes in *W* in each female during the assessed period (Supplementary Table S1), we can observe differences in the direction of the trend line, that is, it can rise (10 geese), fall (5 geese), and, sometimes, remain unchanged (5 geese). However, in addition to this, even lines of the same type had different angles of slope, i.e., different change dynamics of this parameter. Using this unevenness, we attempted to estimate how much the slope angle (TAN*W*) of changes in *W* for the eggs of each female would help us in predicting changes in the sex bias magnitude of the embryos they produced. To achieve a more flexible evaluation of the results obtained, we singled out the trend lines characteristic of the growth and decline of changes in *W* into separate categories, calling them, respectively, “drop” and “growth”. As a result, the respective dependencies were obtained as shown in Figure 6.

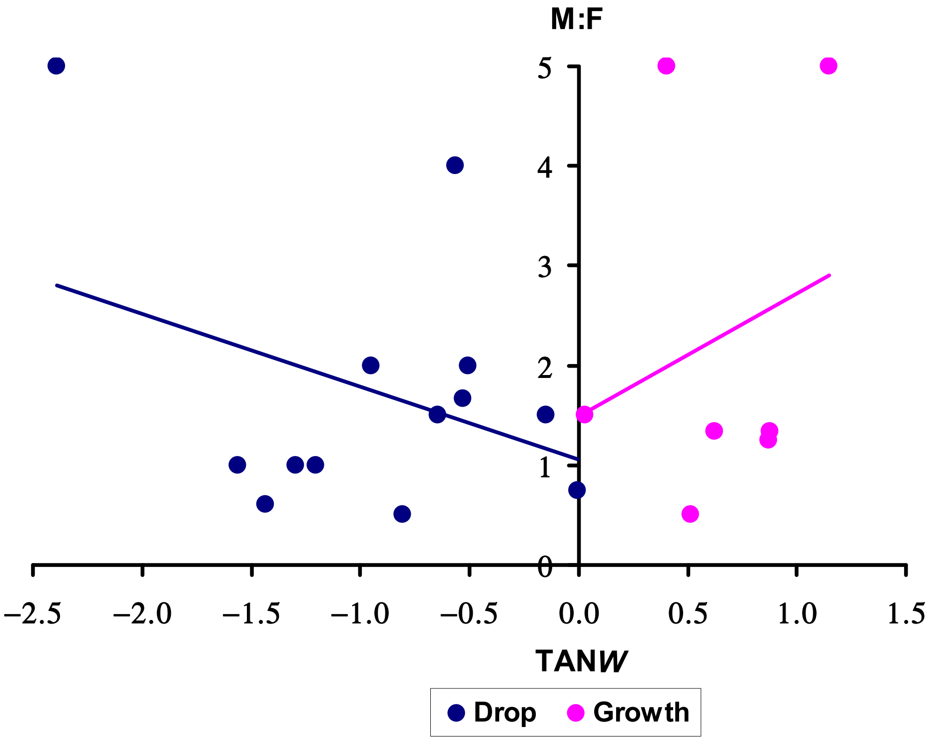

**Figure 6.** Relationship between the slope of the trend line (TAN*W*) that reflected changes in egg weight (*W*) and the sex ratio of goose embryos (M:F).

### *3.6. Power of Synergy Model*

In the previous subsections, we demonstrated several parameters characteristic of each female goose, based on which, with some degree of probability, it was possible to predict a possible change in the sex ratio of embryos in the eggs laid during the observed egg production period. Nevertheless, the probability of such a prediction, which was quite high for some individuals, gave very inaccurate results for others. However, the fact remains that *W* of laid eggs, the tendency of the sex ratio change during lay, and the embryo sex of the first egg laid can be effective criteria for assessing the M:F value. To reach a more reliable prediction of results, we used the power of the synergy effect by including all three analyzed parameters together in the prediction model. These three parameters were *W* of laid eggs, the tendency of its change during lay (TAN*W*), and the embryo sex of the first egg laid (*Em*). Conventionally, data on the embryo sex of the first egg were designated 1 if it was a male, and 2 if it was a female. Before proceeding to the approximation of the data by the mathematical model, their multicollinearity was tested. According to Kutner et al. [41], a strong correlation (near +1 or −1) between variables is a sign of multicollinearity. In this regard, we estimated the Pearson correlation coefficient (*R*) between the parameters *W*, TAN*W*, and *Em*. The following results were obtained: for the pair *W* and TAN*W*, $R = -0.333$, for *W* and *Em*, $R = -0.071$, and for TAN*W* and *Em*, $R = -0.091$. Herewith, all *R* values were insignificant. Thus, the values used to obtain the prediction formula were independent of each other.

The approximation of the experimental data was conducted by a linear regression equation:

$$\text{M:F} = 7.996 - 0.025W - 0.092\text{TAN}W - 0.997Em, \quad (2)$$

where M:F is the dimensionless value of the males to females ratio from one goose during the assessed period; *W* is mean egg weight from one goose (in g); TAN*W* is the slope of the trend line plotted according to *W* data from one goose (in rad); and *Em* is the embryo sex in the first egg laid from the corresponding goose (1 if male and 2 if female). Herewith,

$W$ of those eggs in which the embryo sex could not be identified was not considered during the analysis.

Usage of Equation (2) for computations demonstrated the correlation coefficient between the calculated and true data at the level of $R = 0.327$, although this value was not significant. Consequently, we decided to increase the degree of the regression equation. Understanding that such equations are extremely difficult to explain from the viewpoint of biological processes, the task that we set for ourselves was, nevertheless, to test the possibility of increasing the prediction accuracy based on the synergistic effect of the initial parameters. As a result of mathematical processing of the experimental data, the most accurate result was obtained using a second-order polynomial:

$$\begin{aligned}\mathrm{M:F} = {} & 50.878 - 0.601W - 6.816\mathrm{TAN}W + 34.338Em + 0.044W \cdot \mathrm{TAN}W - 0.202W \cdot Em + \\ & 19.612\mathrm{TAN}W \cdot Em - 0.107W \cdot \mathrm{TAN}W \cdot Em + 0.002W^2 + 0.336(\mathrm{TAN}W)^2 + 1.257Em^2\end{aligned} \tag{3}$$

with $R = 0.724$ ($p < 0.05$).

Undoubtedly, the resultant Equation (3) cannot be used as a definitive tool for practical prediction of the M:F ratio. With this equation, we merely wanted to demonstrate the possible nonlinearity of multiple relationships between parameters, as well as the practical potential of using these parameters in quantitative prediction of the sex ratio.

### *3.7. Randomness Tests*

Assessing biological series for non-randomness is quite complex. This is due to several factors, including the following:

- The sample size is small, which is typical for our case due to the limited number of geese and analytical capabilities.
- A high level of natural variability (genetics, environment, nutrition, stress, etc.) influences the result, which creates strong so-called "biological noise".
- The nonlinearity of parametric relationships, confirmed by the obtained dependence (3). Traditional statistical methods are good at assessing linear relationships. However, if the pattern is nonlinear, it is difficult to capture using standard statistics tools.
- Many tests require normal distribution. In biological research, data often violate these conditions.
- High probability of false negatives, which happens quite often with small effects and high noise levels.

In this regard, the implementation of the randomness verification procedure was examined more closely. To assess the degree of randomness of the estimated series of embryo sexes in laid eggs, that in our case consisted of 74 ones and 54 twos, we tried to generate a similar, but random series, also consisting of ones and twos. As a system for generating random numbers, we attempted to use the algorithm built into the MS Excel program. However, given several skeptical comments about the degree of randomness of such generation [42,43], we also used the classical method of removing balls from a closed, opaque container. To do this, we prepared two identical balls, on one of which the number 1 was written, and on the second 2. By mixing thoroughly and blindly choosing one of the balls, we generated another random row consisting of ones and twos. Thus, a comparison of the series of sex characteristics of goose embryos was carried out with two random series, conventionally called by us 'Excel' and 'balls'. For complete consistency, in the generated random series we did not consider the eggs (seven pieces) that turned out to be unfertilized in the actual dataset of goose eggs. The main comparative characteristics of each of the data series are presented in Table 2.

**Table 2.** Data from actual goose embryo sex series compared to generated random numbers.

| Parameters | Actual Data | Random Excel | Random Balls |
|---|---|---|---|
| Males total | 74 | 61 | 64 |
| Females total | 54 | 67 | 64 |
| Average males per a goose (±SD) | 3.7 ± 1.0 | 3.1 ± 1.8 | 3.2 ± 1.2 |
| Average females per a goose (±SD) | 2.7 ± 1.2 | 3.4 ± 1.7 | 3.2 ± 1.2 |
| Sex ratio (±SD) | 1.37 ± 1.53 $^{a}$ (58:42) | 0.91 ± 2.15 $^{b}$ (48:52) | 1.00 ± 0.82 $^{a,b}$ (50:50) |

$^{a,b}$ Identical superscripts indicate significant differences ($p < 0.05$); the absence of a corresponding index indicates that the differences are insignificant.

Analyzing Table 2, one can conclude that we are not dealing with a random process of sex bias. Obviously, the primary sex of geese, after all, does not conform to the classic 50:50 ratio. At least, randomly generated values showed results closer or even completely corresponding to this ratio (0.91 and 1.00) than laid goose eggs (1.37). Hereby, the difference between the Actual data and Random balls groups was statistically significant. However, the difference between the values of the Random Excel and Random balls groups demonstrated similar significance. If we increased the confidence interval, the difference between the ratios of males to females for the Actual data and Random Excel groups conformed to $p < 0.15$, and for the Actual data and Random balls groups to $p < 0.2$. On the other hand, with an individual analysis of the embryo sex in the eggs for each female, no significant differences could be established between actual and virtual individuals. Furthermore, if we placed them in increasing order of the sex ratio in the clutch, conventionally designating the geese by serial numbers from 1 to 20, the resulting graphical dependencies (Figure 7) were sufficiently similar.

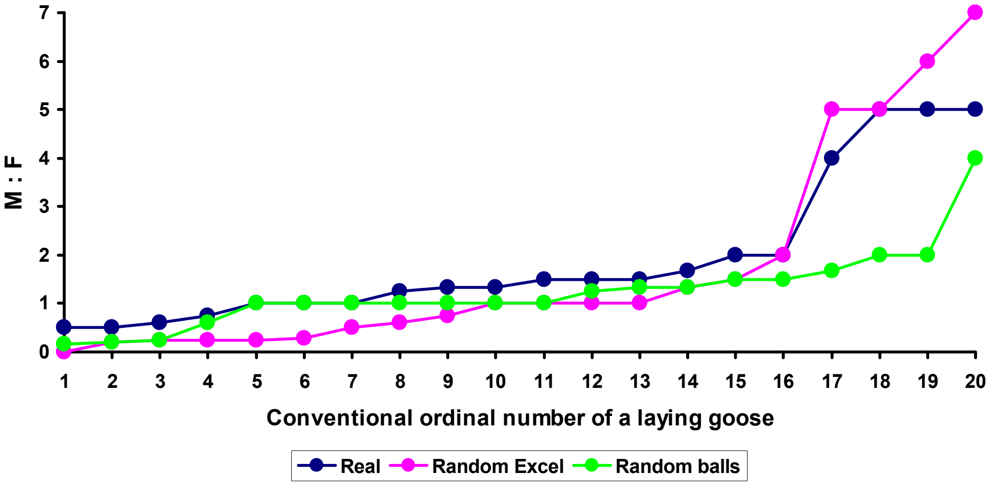

**Figure 7.** The male-to-female ratios between virtual laying geese for actual and random data ranges.

Since it was not possible to draw an unambiguous conclusion, we paid more attention to statistical confirmation of the sex bias reality. A possible candidate for an alternative method to determine significance may be Pearson's [44] $\chi^2$ goodness-of-fit test. This is because the classical purpose of this Pearson's criterion is precisely to assess how actual values differ from theoretical probabilistic analogs [45,46]. In our case, the theoretical probability of sex distribution is 50:50, i.e., the M:F ratio of 0.5 for each goose. However, the calculation of the $\chi^2$ value for the M:F values shown in the graphical dependences in Figure 7 suggested that the data for all three options, both for actual geese and their virtual analogs, differed from the theoretical probability of 50:50 with $p < 0.05$. Herewith, the Random balls value was identical to the theoretical random distribution, compared to the other two series.

We also decided to double-check the adequacy of the obtained differences between the three datasets and the theoretically expected ratio (50:50) using the G-test. In principle, it is similar to the $\chi^2$ test; however, it is more often used in biometric analysis, and therefore may be more suitable for such cases [47]. As expected, the G-test demonstrated similar results: for all three obtained numerical series, the M:F ratio significantly differed from the theoretical probability of 50:50 (at $p < 0.05$).

Using Fisher's [48] exact test, comparison of the Actual data series with the Random balls series demonstrated significant difference at $p < 0.05$. The probability level for the difference between the Actual data and Random Excel values was slightly lower than $p < 0.1$.

We also applied a nonparametric statistical test known as the Wald–Wolfowitz runs test [49,50] that tests the hypothesis of randomness for two data sequences of equal length. This test analyzes the so-called *runs*, i.e., a segment of the sequence consisting of adjacent equal elements. As a result of implementing the Wald–Wolfowitz runs test, all analyzed parameters, i.e., the numerical series (a set of ones and twos), the M:F ratio, the number of males and, accordingly, females, were identified as datasets of random variables with a significance level of $p < 0.05$.

## 4. Discussion

Here, we show that sex ratio in geese begins very male-biased but shifts towards a more female bias over time. In other words, the geese tend to invest most in male offspring early in the laying season. For the samples in the present pilot study (Supplementary Figure S1), it can be noted that over 24 days of the egg-laying, there was a tendency for a slight elevation in the number of female embryos (Supplementary Figure S1a). Moreover, judging by the fact that, by the end of the observation period, the trend line only approached the average value between these indicators (1.5), we can report a pronounced predominance of males over females. The trend in $W$ changes was the opposite of what one would expect in this seasonal breeder as resources become depleted, and over the laying season the investment in egg weight declined, reflecting the reducing energy reserves of the goose over time. That is, the $W$ value gradually reduced towards the end of the assessed period (Supplementary Figure S1b). These findings agreed with those of Salamon and Kent [28], who studied the weight of eggs laid by geese of the same breed group kept under the same conditions as we investigated here. Several studies conducted on other goose breeds also demonstrate a similar trend [32,51–57]. However, a large scatter of parameters and low, insignificant coefficients of determination for both trend lines ($R^2 = 0.014$ in Supplementary Figure S1a; and $R^2 = 0.028$ in Supplementary Figure S1b) did not allow us to draw unambiguous conclusions regarding the obtained trends for changes in these parameters.

We were more successful in addressing the question of whether there was a closer relationship between the embryo sexes and the parameters of their eggs and/or the characteristics of their laying. It was not possible, however, to confirm significance of this relationship in the aggregate analysis of the data produced. In this regard, we tried to use the linear algebraic group theory [40] by executing the appropriate breakdown of the results obtained according to various indicators characteristic of various affine samples of laid eggs. Analysis of the obtained trend lines allowed us to conclude that all geese falling into the group of heavy eggs ($n = 9$) showed a tendency towards a decrease in $W$ by the end of the assessed period or, in extreme cases, towards some constancy of this parameter (Supplementary Figure S2a). At the same time, the geese whose mean $W$ was lower ($n = 11$) were conditionally divided into two subgroups. In one of them (4 geese out of 11), there was a tendency for $W$ to decrease, while in the other (7 geese out of 11), in contrast, the $W$

value grew (Supplementary Figure S2c). As for the M:F ratio, a specific trend could not be detected individually for a particular goose (Supplementary Figure S2b,d).

Despite some obtained information on the relationship between the parameters *W* and M:F, it was not enough to predict unambiguously the magnitude of sex bias in goose embryos. In this regard, we also analyzed other related parameters, including the embryo sex in the first egg. Most likely, this is due to the analogy with wild species. For example, Ankney [58], when studying four-egg clutches of Lesser Snow Geese (*Chen caerulescens caerulescens*), found that males predominated in the first two eggs laid, whereas females predominated in the final two. That author put forward few hypotheses for discussion as to why such a disproportion was possible. In our case, domestic geese were no different from their wild counterparts and followed similar patterns of embryo sex in the first egg [58].

Another appropriate parameter to check was the dynamics of change in *W* during the egg-laying period. Analysis of the obtained graphical dependencies (Figure 6) demonstrated that, for geese that had a drop in *W* during the observed period of egg production, the lower rate of such a decrease (up to constant values of *W*) coincided with a similar decline in the proportion of males in the eggs laid. At the same time, birds that demonstrated a growth in the *W* value had an increased proportion of female embryos, along with a parallel increase in the *W* growth dynamics of laid eggs.

In Sections 3.2–3.5, we explored several single parameters characteristic of each female goose, based on which, with some degree of probability, we were able to predict a possible change in the sex ratio of embryos in the eggs laid during the estimated period of egg production. Nevertheless, the probability of such a prediction, which was high for some individuals, gave somewhat inaccurate results for others. However, the three examined parameters, i.e., *W* of laid eggs, the tendency of its change during lay, and the embryo sex of the first egg laid, can be effective criteria for assessing the M:F value. Using the power of synergy principle, we inferred the appropriate formula (Equation (3)) that combined the above three parameters. Thus, if we assume that the trends we observed during the estimated period at the beginning of laying (24 days) were characteristic of the entire egg-laying cycle of the geese, Equation (3) can be an effective tool for predicting reliable information about the ratio of males to females among all eggs laid. Although the accuracy has been improved compared to the single parameter approach, it is still far from possible practical application. To a greater extent, this approach demonstrates the possibility of its use in similar studies and can also be suitable for analyzing the degree of interaction of selected influencing indicators.

Considering our findings with a degree of skepticism, it might be appropriate to question the fundamental premise that a primary sex ratio bias indeed naturally exists in geese. Were those authors right who state that the primary sex in bird eggs with a sufficiently large sample has a 50:50 ratio, thus adhering to Fisher's [59] theory of equal investment? Was perhaps the series of embryo sexes we obtained purely random? For this purpose, we implemented specific tests for statistical significance of the data produced in this pilot study. The Pearson's goodness-of-fit $\chi^2$ test, as well as the G-test, were not entirely suitable for the conditions of our experiment, since the generated series of random data, despite their "randomness," differed from theoretical randomness, according to which all geese must strictly observe the rule of equal sex ratio in laid eggs. This may be due to the inaccuracy of providing significant results using Pearson's criterion for relatively small samples. In this case, Fisher's [48] exact test is more suitable (e.g., [60]). Another advantage of Fisher's criterion is the fact that it adequately evaluates not only the differences between the empirical and theoretical samples, but also between two independent data series.

Notably, generating a series of random data using MS Excel cannot ensure absolute randomness, and this seems to be the case with most common software. For example, L'Ecuyer [43], testing some random number generators available in popular software environments such as Microsoft Excel, Visual Basic, SUN Java, etc., concluded that they all fail the tests by a wide margin. Nevertheless, when analyzing data on the number of males and females in different samples, we were able to establish significant difference ($p < 0.05$) according to Fisher's test for comparing Actual data with the Random balls series, while comparison with the Random Excel series resulted in a probability of significance at $p < 0.1$.

In order to completely eliminate possible questions about the random or non-random distribution of sexes in goose eggs, we applied the Wald–Wolfowitz runs test [49,50]. This test is used when it is necessary to check the randomness of the data distribution in a sequence that in our case was a numerical series consisting of ones (males) and twos (females). In fact, there are several limitations, amendments, and claims pertaining to this method of testing randomness, as well as, probably, to any other test criterion [61,62]. We chose, however, the Wald–Wolfowitz runs test since it operates with only two values (+ and −), relevant in our case, and is associated with the tabulated values of Student's *t*-test, which already made it possible to identify some differences between our data series.

The choice of a lower probability level, $p < 0.2$, made it possible to assume the non-random nature of the number of females obtained in the series of actual data. Perhaps this could give some hope for non-randomness if, at the same significance level ($p < 0.2$), the series of values consisting of the obtained males (in the obviously random data generated using Excel) failed to pass the test for the presence of a certain pattern. Perhaps, indeed, generating random data series using Excel spreadsheets does not allow us to achieve the principle of absolute randomness. From our point of view, however, the reason is also that, perhaps for this case, it is necessary to introduce such a term as *biased randomness*. It is likely that several other factors have some influence on the random process of sex ratio. Hereby, the strength of the influence of these factors is sufficient to shift the classic 50:50 to one sex or the other but is not sufficient to fix this shift as some kind of stable and significant functional dependence.

From an evolutionary, functional, and behavioral ecology perspective, we described here a significant observation and important data on sex ratio bias in domestic geese. Early in the laying cycle, the geese produce more males, when in good body condition, in accordance with the Trivers–Willard model [13,26]. The question for us is how this is achieved. Our results allowed us to formulate several hypotheses that will help optimize the conduct of experiments in the field of avian sex bias research. Aside from being of academic interest, zoos and captive breeding programs can greatly benefit from research on sex ratios [63]. Another major economic impact of the sex ratio skew in poultry eggs is also possible, since producers prefer to obtain only males or only females, depending on the further use of poultry products (e.g., [64–68]).

## 5. Conclusions

During the present pilot study on sex bias in domestic geese, the following relevant and important observations were made:

1. During the first month of lay, there was a predominance of males over females at the level of 37%. Over time, a gradual reduction in this prevalence of males was observed.
2. Over the period of observation, a gradual decline in *W* was found. Herewith, there was a tendency to increase the number of females in lighter eggs (whose *W* was below the average), and, on the contrary, males in heavier eggs (with *W* above average).
3. A total of 13/16 (~80%) geese had males as the first embryo. The preference toward a male embryo in the first-hatched egg tended to produce more females at the end of laying.

4. More pronounced dynamics of changes in *W* (both its growth and drop) conformed to eggs in which male embryos predominated. A more uniform distribution of *W* during the laying cycle was characteristic of the sex ratio bias towards females.
5. The combination of three parameters characterizing the egg-laying process in geese (i.e., *W*, the dynamics of its change, and the embryo sex in the first egg) enabled us to derive a calculation equation to predict the sex ratio during the observation period of 24 days quite accurately ($R$ = 0.724).
6. The method we used to statistically compare the degree of randomness of the obtained series of data on the embryo sex in goose eggs enabled us to conclude that there was some probability for signatures of sex bias towards the predominance of males. At the same time, generating a random series by the method of blindly selecting the virtual male or female balls turned out to be preferable than using the respective software built in MS Excel.

**Supplementary Materials:** The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/poultry4040059/s1, Table S1: Goose egg data for the laying period from 10 February to 5 March that corresponds to the laying period from Day 10 to Day 33; Table S2: Linear regressions of the trend lines on Supplementary Figure 7 for each goose; Figure S1: Trends in (a) embryonic sex, i.e., males (M = 1) vs. females (F = 2), and (b) egg weight (*W*) during the 24-day egg-laying period of geese; Figure S2: Trends in egg weight, *W* (a,c), and embryo sex ratio (b,d) during the 24 days of the estimated egg-laying period with the corresponding breakdown of geese into two groups laying heavy (a,b) and light (c,d) eggs; Figure S3: Trends in the sex of embryos (M = 1 and F = 2) during the 24-day egg-laying period, assessed depending on whether the first egg laid was male (a) or female (b).

**Author Contributions:** Conceptualization, methodology, software, formal analysis, resources, data curation, visualization, V.G.N.; validation, M.N.R., D.K.G., S.K., A.S., C.O. and J.P.K.; investigation, V.G.N., S.K., A.S., S.C. and J.P.K.; writing—original draft preparation, V.G.N. and A.S.; writing—review and editing, V.G.N., M.N.R., D.K.G., S.K., A.S., C.O. and J.P.K.; supervision, D.K.G.; project administration, M.N.R., D.K.G. and A.S. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding.

**Institutional Review Board Statement:** The study was conducted in accordance with the Declaration of Helsinki and approved by the Department of Agriculture, Fisheries and the Marine (DAFM), Government of Ireland, who provides the DAFM health certificate under EU regulation No. 1069/2009, dated 21 October 2009. The research was in line with the requirements of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (ETS No. 123, Strasbourg, 1986). Only the manipulation of eggs was performed for this pilot study; no direct experiments on animals were used.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** The dataset analyzed during the current pilot study is available in the Figshare repository, 10.6084/m9.figshare.30715265.

**Acknowledgments:** Many thanks to the Department of Agriculture, Fisheries and the Marine (DAFM), Government of Ireland, the Veterinary Inspector Frank Ennett (DAFM) for issuing the health certificate, the Wicklow Calf Company, Killahurler, Arklow, Co. Wicklow for providing transport for the eggs from Ireland to Germany, and their driver, Sean Murray.

**Conflicts of Interest:** Authors John P. Kent and Sara Copeland were employed by the company Ballyrichard House (Farm); author John P. Kent is the owner of this company. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## References

1. Hardy, I.C.W. Possible factors influencing vertebrate sex ratios: An introductory overview. *Appl. Anim. Behav. Sci.* **1997**, *51*, 217–241. [CrossRef]
2. Batellier, F.; Marchal, F.; Scheller, M.F.; Gautron, J.; Sellier, N.; Taouis, M.; Monbrun, C.; Vignal, A.; Brillard, J.P. Sex ratios in mule duck embryos at various stages of incubation. *Theriogenology* **2004**, *61*, 573–580. [CrossRef]
3. Emam, A.M.; Semida, D.A.; El-Full, E.A.; Mahmoud, B.Y.; Abdel-Azim, A.M.; Elnesr, S.S. Is the sex ratio of Japanese quail offspring equal? *Trop. Anim. Health Prod.* **2024**, *56*, 384. [CrossRef]
4. Li, W.M.; Feng, Y.P.; Zhao, R.X.; Fan, Y.Z.; Affara, N.A.; Wu, J.J.; Fang, J.; Tong, Q.; Wang, C.; Zhang, S.J. Sex ratio bias in early-dead embryos of chickens collected during the first week of incubation. *Poult. Sci.* **2008**, *87*, 2231–2233. [CrossRef] [PubMed]
5. Lehikoinen, A.; Öst, M.; Hollmén, T.; Kilpi, M. Does sex-specific duckling mortality contribute to male bias in adult Common Eiders. *Condor* **2008**, *110*, 574–578. [CrossRef]
6. Eiby, Y.A.; Wilmer, J.W.; Booth, D.T. Temperature-dependent sex-biased embryo mortality in a bird. *Proc. R. Soc. B* **2008**, *275*, 2703–2706. [CrossRef]
7. Wu, J.J.; Li, W.M.; Feng, Y.P.; Zhao, R.X.; Wang, C.; Yu, Y.; Yang, L.G.; Zhang, S.J. Sex-biased mortality analysis in chick embryos during the entire period of incubation. *J. Appl. Poult. Res.* **2012**, *21*, 508–512. [CrossRef]
8. Székely, T.; Liker, A.; Freckleton, R.P.; Fichtel, C.; Kappeler, P.M. Sex-biased survival predicts adult sex ratio variation in wild birds. *Proc. R. Soc. B* **2014**, *281*, 20140342. [CrossRef]
9. Payevsky, V.A. Sex ratio and sex-specific survival in avian populations: A review. *Biol. Bull. Rev.* **2021**, *11*, 317–327. [CrossRef]
10. Washaya, S.; Ngorio, U.; Mudzengi, C.P. Effects of the breeding season, birth type and sex on growth and reproductive performances of sheep breeds. *Trop. Anim. Health Prod.* **2023**, *55*, 188. [CrossRef]
11. Aktaş, A.H.; Dursun, Ş.; Doğan, Ş.; Kiyma, Z.; Demirci, U.; Halıcı, I. Effects of ewe live weight and age on reproductive performance, lamb growth, and survival in Central Anatolian Merino sheep. *Arch. Anim. Breed.* **2015**, *58*, 451–459. [CrossRef]
12. Karlin, S.; Lessard, S. *Theoretical Studies on Sex Ratio Evolution*; Princeton University Press: Princeton, NJ, USA, 2020.
13. Douhard, M. Offspring sex ratio in mammals and the Trivers-Willard hypothesis: In pursuit of unambiguous evidence. *BioEssays* **2017**, *39*, 1700043. [CrossRef]
14. Quaresma, M.; Rodrigues, M.; Medeiros-Sousa, P.; Martins, A. Calf-sex bias in Holstein dairy milk production under extensive management. *Livest. Sci.* **2020**, *235*, 104016. [CrossRef]
15. Casellas, J.; Vázquez-Gómez, M.; Id-Lahoucine, S.; de Hijas-Villalba, M.M. Evidential segregation analysis for offspring sex ratio in rabbit and sheep populations. *Livest. Sci.* **2023**, *277*, 105354. [CrossRef]
16. Pike, T.W.; Petrie, M. Potential mechanisms of avian sex manipulation. *Biol. Rev.* **2003**, *78*, 553–574. [CrossRef] [PubMed]
17. Alonso-Alvarez, C. Manipulation of primary sex-ratio: An updated review. *Avian Poult. Biol. Rev.* **2006**, *17*, 1–20. [CrossRef]
18. Zakaria, A.H.; Omar, O.H. Egg laying pattern, egg weight, body weight at hatch, and sex ratio bias relative to oviposition time of young-and mid-age broiler breeders. *Anim. Reprod. Sci.* **2013**, *141*, 80–85. [CrossRef] [PubMed]
19. Aslam, M.A. Offspring Sex Ratio Bias and Sex Related Characteristics of Eggs in Chicken. Ph.D. Thesis, Wageningen University, Wageningen, The Netherlands, 2014.
20. Kutnyuk, P.I.; Bondarenko, Y.V.; Ivchenko, V.V. The Formation of the Secondary Sex Ratio in Goose Populations from the Viewpoint of Catastrophe Theory. In Proceedings of the Genetics of Animal Productivity, Abstracts of the All-Ukrainian Scientific and Practical Conference, Dedicated to the 90th Anniversary of the Outstanding Scientist, One of the Patriarchs of Genetics, Prof. M.M. Kolesnyk, Kyiv, Ukraine, 20–21 December 1994; n.p.: Kyiv, Ukraine, 1994; p. 37. Available online: https://scholar.google.com/citations?view_op=view_citation&citation_for_view=c1PnDYEAAAAJ:WA5NYHcadZ8C (accessed on 23 July 2025).
21. Kutnyuk, P.I.; Bondarenko, Y.V.; Ivchenko, V.V. Nature of Goose Secondary Sex Ratio in View of Adaptive Models of Catastrophe Theory. In Proceedings of the 11th International Symposium on Current Problems in Avian Genetics, Balice near Kraków, Poland, 29 May–1 June 1995; World's Poultry Science Association, Polish Branch: Balice near Kraków, Poland, 1995; pp. 208–211. Available online: https://scholar.google.com/citations?view_op=view_citation&citation_for_view=c1PnDYEAAAAJ:PELIpwtuRlgC (accessed on 23 July 2025).
22. Kutnyuk, P.I.; Bondarenko, Y.V.; Ivchenko, V.V. Priroda vtorichnogo sootnosheniya polov u gusey s tochki zreniya adaptivnykh modeley teorii dinamicheskikh sistem [The nature of the secondary sex ratio in geese from the viewpoint of adaptive models in the theory of dynamic systems]. *Ptakhivnytstvo [Poult. Farm.]* **1999**, *48*, 18–22.
23. Komdeur, J.; Pen, I. Adaptive sex allocation in birds: The complexities of linking theory and practice. *Philos. Trans. R. Soc. B Biol. Sci.* **2002**, *357*, 373–380. [CrossRef]
24. Rutkowska, J.; Badyaev, A.V. Meiotic drive and sex determination: Molecular and cytological mechanisms of sex ratio adjustment in birds. *Philos. Trans. R. Soc. B Biol. Sci.* **2008**, *363*, 1675–1686. [CrossRef]
25. Narushin, V.G.; Romanov, M.N.; Klein, S.; Salamon, A.; Manzoni, G.; Kent, J.P. Meteorological dependencies in ducks: Influence of weather on sex ratio bias and hatchability. *Res. Vet. Sci.* **2025**, *submitted*.

26. Schindler, S.; Gaillard, J.M.; Grüning, A.; Neuhaus, P.; Traill, L.W.; Tuljapurkar, S.; Coulson, T. Sex-specific demography and generalization of the Trivers–Willard theory. *Nature* **2015**, *526*, 249–252. [CrossRef]
27. Kent, J.P.; Murphy, K.J. Synchronized egg laying in flocks of domestic geese (*Anser anser*). *Appl. Anim. Behav. Sci.* **2003**, *82*, 219–228. [CrossRef]
28. Salamon, A.; Kent, J.P. Egg weight declines to baseline levels over the laying season in domestic geese (Anser anser domesticus). *Int. J. Poult. Sci.* **2013**, *12*, 509–516. [CrossRef]
29. Shi, Z.D.; Tian, Y.B.; Wu, W.; Wang, Z.Y. Controlling reproductive seasonality in the geese: A review. *Worlds Poult. Sci. J.* **2008**, *64*, 343–355. [CrossRef]
30. Boonstra, T.A.; Clark, M.E.; Reed, W.L. Maternal resource variation across the laying sequence in Canada geese *Branta canadensis maxima*. *J. Avian Biol.* **2009**, *40*, 520–528. [CrossRef]
31. Krist, M. Egg size and offspring quality: A meta-analysis in birds. *Biol. Rev. Camb. Philos. Soc.* **2011**, *86*, 692–716. [CrossRef] [PubMed]
32. Karadağ, S.; Kırmızıbayrak, T. Egg production and traits and hatching traits of geese in the first and next laying seasons. *Indian J. Anim. Res.* **2024**, BF-1897. [CrossRef]
33. Fridolfsson, A.K.; Ellegren, H. Molecular evolution of the avian *CHD1* genes on the Z and W sex chromosomes. *Genetics* **2000**, *155*, 1903–1912. [CrossRef]
34. Turcu, M.-C.; Pașțiu, A.I.; Bel, L.-V.; Pusta, D.L. Minimally invasive sampling methods for molecular sexing of wild and companion birds. *Animals* **2023**, *13*, 3417. [CrossRef]
35. Romanov, M.N.; Ellegren, H.; Dodgson, J.B. Polymerase Chain Reaction Amplified Markers for Bird Sexing. In Proceedings of the International Plant and Animal Genome IX Conference, San Diego, CA, USA, 13–17 January 2001; Scherago International: San Diego, CA, USA, 2001. Abstract P229. p. 118. Available online: https://web.archive.org/web/20040624180938/http://www.intl-pag.org/pag/9/abstracts/P3a_14.html (accessed on 24 June 2004).
36. Romanov, M.N.; Betuel, A.M.; Chemnick, L.G.; Ryder, O.A.; Kulibaba, R.O.; Tereshchenko, O.V.; Payne, W.S.; Delekta, P.C.; Dodgson, J.B.; Tuttle, E.M.; et al. Widely applicable PCR markers for sex identification in birds. *Russ. J. Genet.* **2019**, *55*, 220–231. [CrossRef]
37. Tagliavia, M.; Catania, V.; Dell'Omo, G.; Massa, B. High-performance PCR for alleles discrimination of chromo-helicase-DNA binding protein (CHD1) gene in bird sexing. *Biology* **2023**, *12*, 300. [CrossRef]
38. Altgilbers, S.; Klein, S.; Dierks, C.; Weigend, S.; Kues, W.A. Cultivation and characterization of primordial germ cells from blue layer hybrids (Araucana crossbreeds) and generation of germline chimeric chickens. *Sci. Rep.* **2021**, *11*, 12923. [CrossRef] [PubMed]
39. Cochran, W.G. *Sampling Techniques*, 3rd ed.; John Wiley & Sons Inc.: New York, NY, USA, 1977. Available online: https://educationexclusive.com/upload/pdf/Sampling%20Techniques-William%20G.%20Cochran.pdf (accessed on 23 July 2025).
40. Borel, A. Borel subgroups; reductive groups. In *Linear Algebraic Groups. Graduate Texts in Mathematics*; Springer: New York, NY, USA, 1991; Volume 126, pp. 147–202. [CrossRef]
41. Kutner, M.H.; Nachtsheim, C.J.; Neter, J.; Li, W. *Applied Linear Statistical Models*, 5th ed.; McGraw Hill Irwin: New York, NY, USA, 2005; pp. 213–508. Available online: https://web.archive.org/web/20231117053807/https://thuvienso.hoasen.edu.vn/bitstream/handle/123456789/9564/Contents.pdf (accessed on 17 November 2023).
42. L'Ecuyer, P. Random number generation. In *Handbook of Simulation: Principles, Methodology, Advances, Applications, and Practice*; Banks, J., Ed.; John Wiley & Sons Inc.: New York, NY, USA, 1998; pp. 93–137. [CrossRef]
43. L'Ecuyer, P. Software for Uniform Random Number Generation: Distinguishing the Good and the Bad. In Proceedings of the 2001 Winter Simulation Conference (Cat. No. 01CH37304), Arlington, VA, USA, 9–12 December 2001; IEEE: Arlington, VA, USA, 2001; Volume 1; pp. 95–105. [CrossRef]
44. Shevlyakov, G.L.; Oja, H. *Robust Correlation: Theory and Applications*; John Wiley & Sons Inc.: New York, NY, USA, 2016. [CrossRef]
45. Alon, N.; Spencer, J.H. *The Probabilistic Method*, 4th ed.; John Wiley & Sons Inc.: New York, NY, USA, 2016; Available online: https://www.wiley.com/en-us/The+Probabilistic+Method%2C+4th+Edition-p-9781119061953 (accessed on 23 July 2025).
46. McHugh, M.L. The Chi-square test of independence. *Biochem. Med.* **2013**, *23*, 143–149. [CrossRef] [PubMed]
47. McDonald, J.H. G–test of goodness-of-fit. In *Handbook of Biological Statistics*, 3rd ed.; Sparky House Publishing: Baltimore, MD, USA, 2014; pp. 53–58. Available online: https://www.biostathandbook.com/gtestgof.html (accessed on 23 July 2025).

48. Çetinkaya, O. The uses chi square tests family in fisheries and aquaculture studies. In *Pioneer Research in Agriculture, Forest and Water Issues*; Yarpuz Bozdoğan, N., Ed.; All Sciences Academy: Konya, Turkey, 2025; pp. 36–79. Available online: https://www.allsciencesacademy.com/_files/ugd/13252f_c01e267293c6495287adfba575acd29f.pdf#page=38 (accessed on 23 July 2025).
49. De La Rubia, J.M. Calculation of Two-Tailed Exact Probability in the Wald-Wolfowitz One-Sample Runs Test. *J. Data Anal. Inf. Process.* **2024**, *12*, 89–114. [CrossRef]
50. Veretel'nikova, I.V.; Lemeshko, B.Y. The Analytical Review of Tests for Randomness and the Absence of Av Trend. In Proceedings of the 2014 12th International Conference on Actual Problems of Electronics Instrument Engineering (APEIE), Novosibirsk, Russia, 2–4 October 2014; IEEE: New York, NY, USA, 2014; pp. 532–539. [CrossRef]
51. Cüneydioğlu, E.; Erdem, E.; Yalçın, S. Effects of the breeder age on the egg yield and egg quality traits of Landes geese (*Anser anser*). *Trop. Anim. Health Prod.* **2022**, *54*, 387. [CrossRef]
52. Brassó, L.D.; Komlósi, I.; Czeglédi, L.; Gulyás, G. The effect of group size and laying month on the quality, IgG, and corticosterone levels of goose eggs. *Arch. Anim. Breed.* **2024**, *67*, 551–559. [CrossRef]
53. Kucharska-Gaca, J.; Adamski, M.; Biesek, J. The age of the geese from the parent flock and the laying period affect the features of the eggs. *Poult. Sci.* **2022**, *101*, 102094. [CrossRef]
54. Brun, J.M.; Delaunay, I.; Sellier, N.; Alletru, B.; Rouvier, R.; Tixier-Boichard, M. Analysis of laying traits in first cycle geese in two production systems. *Anim. Res.* **2003**, *52*, 125–140. [CrossRef]
55. Mroz, E.; Lepek, G. A biological evaluation of hatches in different phases of goose egg production. *Pol. J. Nat. Sci.* **2003**, *13*, 115–123. Available online: https://agris.fao.org/search/en/providers/122651/records/64723e8608fd68d546002314 (accessed on 23 July 2025).
56. Mazanowski, A.; Adamski, M. The structure, chemical composition and time trends of egg quality characteristics in high-producing geese. *Arch. Geflugelk.* **2006**, *70*, 127–133. [CrossRef]
57. Biesiada-Drzazga, B.; Banaszewska, D.; Koncerewicz, A.; Jóźwik, A.; Horbańczuk, J. Examination of changes in selected external and internal egg traits during the geese laying season and their effect on gosling hatching results. *Eur. Poult. Sci.* **2015**, *79*, 77. [CrossRef]
58. Ankney, C.D. Sex ratio varies with egg sequence in Lesser Snow Geese. *Auk* **1982**, *99*, 662–666.
59. Sharma, M.D.; Wilson, A.J.; Hosken, D.J. Fisher's sons' effect in sexual selection: Absent, intermittent or just low experimental power? *J. Evol. Biol.* **2016**, *29*, 2464–2470. [CrossRef] [PubMed]
60. Kim, H.Y. Statistical notes for clinical researchers: Chi-squared test and Fisher's exact test. *Restor. Dent. Endod.* **2017**, *42*, 152–155. [CrossRef] [PubMed]
61. Ramazanov, S.; Askerbeyli, R. Power Analysis of Mann Whitney, Two Sample Kolmogorov Smirnov and Wald-Wolfowitz Runs Tests: Comparison with Monte Carlo Simulation in the Case of Variance Heterogeneity. EasyChair Preprint 10501, 2023. Available online: https://easychair.org/publications/preprint/f21T (accessed on 23 July 2025).
62. Alhakim, A.; Hooper, W. A non-parametric test for several independent samples. *J. Nonparametr. Stat.* **2008**, *20*, 253–261. [CrossRef]
63. Hays, G.C.; Mazaris, A.D.; Schofield, G.; Laloë, J.O. Population viability at extreme sex-ratio skews produced by temperature-dependent sex determination. *Proc. R. Soc. B* **2017**, *284*, 20162576. [CrossRef]
64. Narushin, V.G.; Romanov, M.N.; Bondarenko, Y.V. Studies on Chick Embryo Sex Determination and Manipulation in the Countries of the Former USSR—A Review. 1. Sex Differences in Eggs. In Proceedings of the 9th European Poultry Conference, Glasgow, UK, 7–12 August 1994; Great Britain, Ministry of Agriculture, Fisheries and Food: London, UK; World's Poultry Science Association, UK Branch: Glasgow, UK, 1994; Volume 1, pp. 320–321. Available online: https://kar.kent.ac.uk/46311/ (accessed on 23 July 2025).
65. Narushin, V.G.; Romanov, M.N.; Bogatyr, V.P. K voprosu o vzaimosvyazi morfologicheskikh parametrov kurinykh yaits s polom tsyplyat [On Relationship between Chicken Egg Morphological Parameters and Chick Sex]. In Proceedings of the Abstracts of the 2nd Ukrainian Poultry Conference, Borky, Ukraine, 14–16 May 1996; World's Poultry Science Association, Ukrainian Branch, Poultry Research Institute, UAAS: Borky, Ukraine, 1996; pp. 92–93. Available online: https://kar.kent.ac.uk/46303/ (accessed on 23 July 2025).
66. Narushin, V.G.; Romanov, M.N.; Bogatyr, V.P. Method for Preincubational Prediction of Embryo Sex in Chicken Eggs. In Proceedings of the 8th World Conference on Animal Production, Seoul, Republic of Korea, 28 June–4 July 1998; Seoul National University: Seoul, Korea, 1998; Volume 1, pp. 832–833. Available online: https://kar.kent.ac.uk/46410/ (accessed on 23 July 2025).

67. Romanov, M.N.; Narushin, V.G.; Sakhatsky, N.I. Studies on Chick Embryo Sex Determination and Manipulation in the Countries of the Former USSR—A Review. 2. Shifting of Sex Ratio. In Proceedings of the 9th European Poultry Conference, Glasgow, UK, 7–12 August 1994; Great Britain, Ministry of Agriculture, Fisheries and Food: London, UK; World's Poultry Science Association, UK Branch: Glasgow, Scotland, UK, 1994; Volume 1, pp. 324–326. Available online: https://kar.kent.ac.uk/46312/ (accessed on 23 July 2025).
68. Trukhina, A.V.; Romanov, M.N.; Smirnov, A.F.; Narushin, V.G. Perspektivy polucheniya osobey preimushchestvenno opredelennogo pola u ptits [Prospects for Producing Individuals of Predominantly a Certain Sex in Birds]. In Proceedings of the Materials of the 3rd International Scientific and Practical Conference on Molecular Genetic Technologies for Analysis of Gene Expression Related to Animal Productivity and Disease Resistance, Moscow, Russia, 30 September 2021; Sel'skokhozyaistvennye tekhnologii: Moscow, Russia, 2021; pp. 80–94. [CrossRef]