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Research Article

The effect of artificial insemination on egg fertilization at different times of nest construction in gloster canaries

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Abstract

When canaries are sexually active, they mate during the breeding season. During this time, the female canary builds a nest. In the days following the completion of nest construction, they lay eggs and incubate. In artificial insemination, the skill of the practitioner and knowing the appropriate artificial insemination time increase the chance of success. In the study, 8 male and 8 female Gloster canaries were used. Artificial insemination practices arranged for each canary inseminated at different times were called groups. Each artificial insemination application was made 2 times at the specified times. It was applied just before Group 1 canary nest construction was completed and when nest construction was finished, Group 2 canary when nest construction was started and nest was completed, Group 3 was applied when nesting material was given and nest was completed. Female canaries were immediately inseminated by cloacal method with semen taken from male breeders at different times of the nest building phase. Among group applications, it was observed that the 3rd group application was significantly successful when compared to the others. The aim of this study is to determine the effects of artificial insemination applied at different times of the nest building process on fertilization in canaries.

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1. Introduction

With the domestication of canaries for hundreds of years, canaries have been bred for their singing and impressive feather characteristics. The breeding season is between spring and summer. The canaries mate during mate selection shortly before the eggs hatch. The egg-laying period of fertile eggs is 13-14 days (Cartwright, 2000). When the hatchlings are 17-25 days old, the female canary is ready to mate again sexually. They consume a lot of energy during nest building and mating behaviors (Beguin et al., 2006). The suitable temperature for production is between 18°C-24°C (Tamura et al., 2021). Light plays an important role in the

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breeding season. Birds that are in an environment of 10 hours of light and 14 hours of darkness in October should be applied with photoperiod lighting by increasing the amount of light regularly, and 16 hours of light and 8 hours of darkness should be applied until July (Ward et al, 2003).During the breeding season, male canaries show signs of estrus due to the increase in testosterone levels and their singing behavior increases significantly (Voight and Leitner, 2008).

It is necessary to feed the canaries rich in protein to prepare them for the breeding season. In addition, the energy requirement increases during the breeding season (Harper and Skinner, 1998). Although female birds do not consume food during the laying period, they meet the need during this period by using the protein and fat reserves in the body (Houston et al., 1995).

The health of the canaries selected for production is very important for production success. Diseases directly related to reproduction can be seen during the breeding season of canaries. Unfortunately, these diseases can cause serious economic losses during production. Various bacterial infections that can develop in the embryonic period and harm the offspring after hatching, as well as embryonic deaths, may adversely affect the production success. Especially *E. Coli*, *Klebsiella*, *Bacillus*, and *Staphylococcus* species have been detected frequently in canaries (Di Francesco et al., 2018). In addition, parasitic infestations of canaries are a big negative. It has been reported that especially *Dermanyssus gallinae* mite causes restlessness due to itching, inability to sleep, decrease in the number of eggs, dermatitis on the skin, weakening and anemia, and death (Circella et al., 2011).

It is vitally important to reduce the stress as much as possible for artificial insemination applied to increase the success of the production season in canaries. In addition, the quality of the semen used for artificial insemination and the method of application should be sufficient. Since increasing the fertile egg rate is also related to the skill of the practitioner, frequent practice will increase success (Blanco et al., 2009). It has been stated that reproductive success in poultry species is directly related to environmental conditions, adaptability to the environment, species-specific behaviors, and success in artificial insemination practices. In addition, it has been reported that the anatomical structure of the species from which semen is taken or artificial insemination, the semen collection technique, the functional quality of the semen specific to the bird species, and the transmission of the sperm that affects the fertilization ability of the egg will increase the success rate of artificial insemination (Gee et al., 2004).

2. Material and Methods

In this study, egg fertility rate was tried to be determined by insemination of female canary at different stages of nest construction. Canaries were selected from young animals 2 years old. 8 male and 8 female Gloster canaries were used in the study. Male and female canaries have never been combined. Nest construction was encouraged by giving nest material after female canary reproductive functions were active.

2.1 Preparing Canaries for the Breeding Season

Eight male and 8 female canaries were used in the study. The canaries were selected from healthy birds 2 years of age. Male and female canaries were evaluated in 60X50X40 cm production cages in a low-stress environment where they could move freely (Figure 1). Starting from January, the light duration was increased with photoperiod application and it was arranged as 16 hours of light and 8 hours of darkness from April to mid-July (Ward et al, 2003). Ectoparasite treatment was applied to the canaries before the breeding period. For this purpose, local Selamectin was applied (Hahn et al., 2014). In addition, male and female canaries were fed with energy and protein-rich diets as the breeding season approached. It is known that especially female canaries benefit from protein and fat reserves in the body during the incubation period (Houston et al., 1995). During the production season, the ambient temperature was between 18-22°C. It has been stated that the required ambient temperature should be 18-24°C (Tamura et al., 2021).

Figure 1. Canary breeding cages (60x50x40 cm dimensions**)**

2.2. Giving Nest Material to Female Canaries and Incubation Process

Females in the same environment as males, but in separate cages, become sexually active by being influenced by the call of active males. Activated female canaries were encouraged to build nests by giving nest materials for internal nesting and nest building (Figure 2). The nest construction phase was completed in 2-6 days, depending on the willingness of the female. Under normal conditions, mates mate during nest building. When the hatchlings are 17 days old, the female starts the nest preparations again (Beguin et al., 2006). However, since there was no male in the cage of the females in the study, this process took up to 25-30 days. Artificial insemination of breeding females was carried out during the nest construction phase. After 25 days after hatching, the nest and materials were given again. The chicks were separated from their parents when they were 30 days old, as they could become self-sufficient. For each female canary, 3 different artificial insemination practices were carried out at 25-day periods throughout the breeding season. Each insemination application was carried out 2 times at the specified times. The first insemination application (Group 1) just before and after the nest construction is completed, the 2nd insemination application (Group 2), when the canary nest construction is started and the nest building is completed, the 3rd insemination application (Group 3) nest material performed when given and when the nest was completed. Egg controls were performed on the 8th day following ovulation and the fertilization rate was evaluated. Females that failed artificial insemination or whose eggs were empty in the controls were kept in incubation until they completed the incubation period of 13-14 days. The aim here is to consider that early termination of incubation may cause stress or hormonal irregularities and adversely affect the subsequent artificial insemination process. The number of eggs, egg occupancy rate, and hatchability of each female canary were noted.

Figure 2. Female canary building a nest

2.3. Semen Collection from Male Canaries

The cloacal massage method was used to collect semen from songbirds (Kucera and Heidinger 2018). The movement of the bird was restricted by holding the feet in the palm. In addition, a suitable position for the application was created. The massage was applied from the base of the cloaca towards the cloacal protuberance by applying light pressure with the help of the thumb and forefinger in the direction of the tail (Figure 3). However, care should be taken as much as possible. Otherwise, contamination of the semen or injury may result. The massage was continued until semen was seen. As soon as semen appeared in light brown, they were collected with the help of a microhematocrit tube. Before starting the artificial insemination practices, the semen of each male canary was evaluated in terms of motility and abnormal spermatozoa, and it was checked whether there was an individual problem in the birds. It was observed that the motility was over 70% and the rate of abnormal spermatozoa was insignificant.

Figure 3. Artificial insemination and revealing protuberance with cloacal massage technique

2.4. Artificial Insemination

It has been reported that semen used in artificial insemination in poultry can reach only about 1% of semen storage tubules (Brillard, 1993). Although there are many artificial insemination methods in poultry, the most commonly used method is the cloacal and intravaginal method. Although the intravaginal method is more successful, it is difficult to cross the vaginal orifice in small birds such as canaries (Blanco et al., 2009). Therefore, the cloacal method, which is an easier and faster method, was preferred in the study as it may cause undesirable situations. Female birds that are sexually active during the breeding season

shed their abdomen feathers (Blanco et al., 2009). This provided convenience during application (Figure 4A). Before artificial insemination, the female bird was flown into the cage and defecated. Thus, the risk of contamination during the application was minimized. A carefully held female bird was stimulated by an abdominal massage before insemination (Blanco et al., 2009). The semen in the microhematocrit tube collected from the male canary was promptly delivered into the cloaca of the female canary (Figure 4 B).

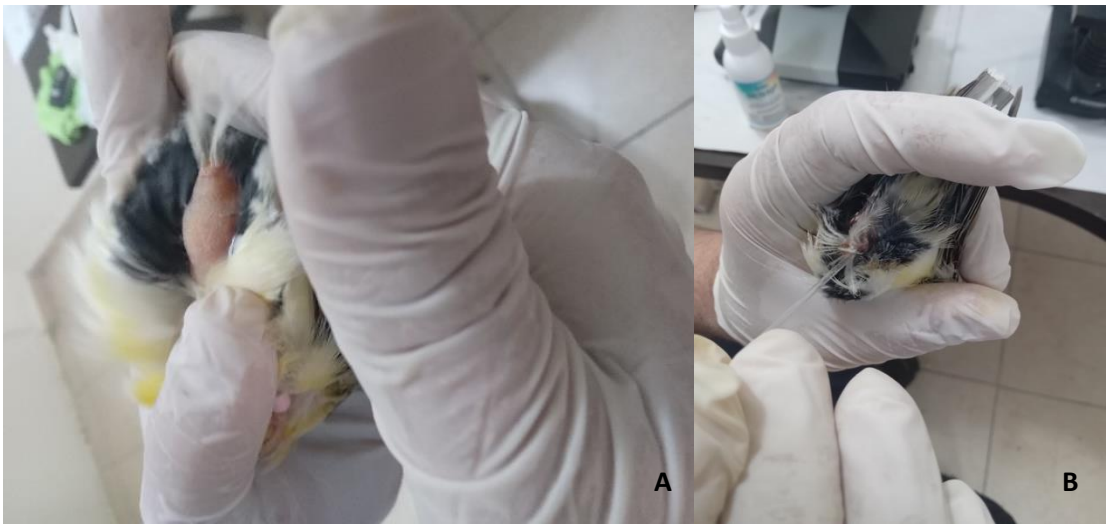

Figure 4. A**:** Active female abdomen feathers shed, B: Artificial insemination technique

2.5. Egg Filling Control and Leaving the Hatchlings

Each female canary was checked for fullness by light on the 8th day following spawning (Figure 5A). Empty eggs were taken and no new nest was given for 25 days for the female to take care of the hatchlings at the end of the incubation period (Figure 5B). It happened in offspring that could not hatch despite fertilization during incubation (Figure 6). Cubs were taken from the female's cage when they were 30 days old. During this time, females were re-seeded according to the procedure established during nest construction.

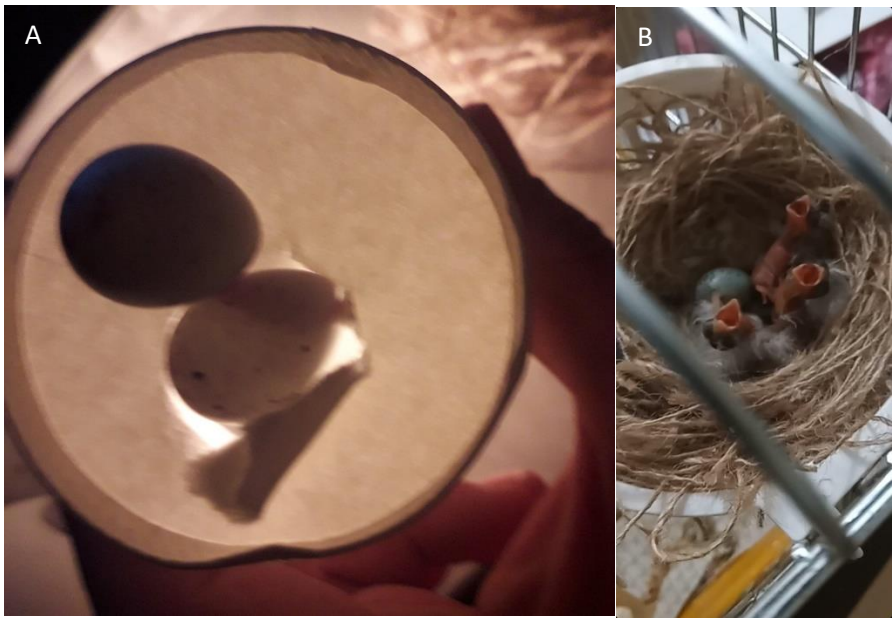

Figure 5. A**:** Empty and full eggs B: Newly hatched chicks

Figure 6. Unable to hatch despite normal development

3. Statistical Analysis

The data obtained in the study were summarized as arithmetic means and standard errors. The statistical significance level for each group used in the study was made with 'One-Way Analysis of Variance'. Duncan's multiple range test was used to investigate the differences between group means. The effects of the groups were evaluated at the $P<0.05$ level. SPSS 20.0 package program was used for variance analysis of the data obtained from the research.

4. Result and Discussion

The canaries have long been widely bred in the world for their beautiful voice and attractive appearance. Spring-summer months are known as the canary breeding season. Some problems that can be seen during mating in canaries may result in low fertility and result in an unsuccessful breeding season for producers. In addition, canaries that are aged or unable to mate due to physical defects, but have quality breeder qualifications, are out of production. Thanks to artificial insemination, negative causes that restrict reproduction can be minimized. However, for this, besides the species-specific breeding conditions, the appropriate artificial insemination time should be known to ensure maximum fertility. In the study, 8 female canaries were inseminated at different times during the nest building phase. Comparing the results regarding the appropriate time for artificial insemination are given in Table 1. There was a statistically significant difference between the groups in terms of egg fertility rates ($P<0.05$). The best fertility rate was found in Group 3. Therefore, according to the results of this study, it can be said that the best insemination time is to be done twice when the nest material is given to the canaries and when the nest construction is completed.

Table 1. Evaluation of egg fertility rate (%) (N=8) (Groups 1, 2, 3: Eggs of inseminated canaries at the beginning, middle and end of nest construction, respectively)

Groups	***Egg Fertility Rate (%)***
Group 1	39.575b
Group 2	29.163b
Group 3	63.538a
SH	5.152
P	**0.013**

a, b: The differences between the means shown with different letters in the same column are significant (P<0.05)

In a study on the subject, it was stated that the most appropriate artificial insemination time for small breed birds should be 3 times 1 week before ovulation (Blanco et al., 2009). In our study, it was observed that the nest building process of canaries lasts 2-6 days and the egg laying process is entered within a few days after the nest construction is completed. Since it is difficult to predict the exact time of ovulation, the nest-building phase has been determined as a guideline. In the study, 2 artificial inseminations were made for each application to provide an example between the groups. In another study, it was stated that the sperm remained in the reproductive canal in chickens for 8-10 days. In addition, it was emphasized that the number of spermatozoa in the reproductive canal was significantly high in 1-6 days (Hemmings et al., 2015). Although the study overlaps with our current study, the fact that the animal material is chicken is seen as a difference. However, the study may answer the question of how long before the time of ovulation artificial insemination should be done in birds. In our study, the best result was when the nest material was given and the nest was completed, but since this process took 2-6 days, it seems that the current study supports our study. Birkhead and Moller (1993) emphasized that semen storage time in poultry is more limited than in reptiles and mammals. However, it has been stated that semen can be stored for at least 8 days in birds. In this respect, the study overlaps with our current study and supports the success of artificial insemination applied when nesting material is given. Since there are not enough resources on artificial insemination in canaries, it is thought that more studies on the subject are needed.

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