

Timing of the Ovariectomy Operation Effects the Survival of Newly-hatched Chicks ^[1]

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Summary

Preliminary experiments performed in our laboratory on orthotopic transplantation of the ovarian tissue in newly-hatched chicks demonstrated that majority of chicks could not survive the surgical technique described by Song and Silversides (2006) and die within 24 h following the operation. A series of experiments were performed in the present study to determine underlying causes of this high mortality rate. A total of 50 newly-hatched chicks (day 0) went through the operation in the first series of experiments. It was determined that the survival rate did not exceed 36% and majority of chicks died within 24 h following the operation in agreement with our preliminary observations. The effect of the removal of the yolk sac on various time points in reference to the day of hatching was examined in a second series of experiments. When the yolk sac was removed on the day of hatching (n=10, day 0), only 30% of the chicks survived the operation. When the yolk sac is removed either on day1 (n=11, 24 h post-hatch) or on day2 (n=8, 48 h post-hatch) 73 and 100% of chicks survived the operation, respectively. These results suggest that the orthotopic transplantation of the ovarian tissue in the chick should be performed one or two days following the day of hatching to avoid the high mortality rate associated with the surgical technique.

Keywords: Chick, Orthotopic, Ovariectomy, Ovary, Transplantation

Overektomi Operasyonu Zamanının Kuluçkadan Yeni Çıkmış Civcivlerin Hayatta Kalma Oranına Etkileri

Özet

Birimimizde kuluçkadan yeni çıkmış olan civcivlerde ortotopik ovaryum transplantasyonu üzerine yapmış olduğumuz ön çalışmalar, Song ve Silversides (2006) tarafından tanımlanan operasyon tekniğinin uygulanması sonucunda operasyon yapılan civcivlerin çoğunluğunun operasyonu izleyen 24 saat içerisinde öldüğünü göstermiştir. Sunulan çalışmada, söz konusu tekniğin uygulanmasına bağlı olarak ortaya çıkan bu yüksek ölüm oranının nedenlerini belirlemek amacıyla bir seri deney gerçekleştirilmiştir. Birinci seri deneylerde toplam 50 adet kuluçkadan yeni çıkmış civcive (0. gün) overektomi operasyonu uygulanmıştır. Elde edilen veriler, yaşama oranının %36'yı geçmediğini ve ön verilerimizi doğrular nitelikte civcivlerin çoğunluğunun operasyonu takiben 24 saat içerisinde öldüğünü göstermiştir. İkinci seri deneylerde, kuluçkadan çıkış günü referans alınarak yumurta sarı kesesinin uzaklaştırılmasının ölüm oranları üzerine olan etkisi incelenmiştir. Yumurta sarı kesesinin kuluçkadan çıkış günü (n=10, 0. gün) uzaklaştırılması durumunda, civcivlerin yalnızca %30'u hayatta kalmıştır. Yumurta sarı kesesinin kuluçkadan çıkışı izleyen 24. saatte (n=11, 1. gün) ya da 48. saatte (n=8, 2. gün) uzaklaştırılması durumunda ise, civcivlerin sırasıyla %70 ve %100'ü hayatta kalmıştır. Bu bulgular, operasyon tekniğine bağlı olarak gelişen yüksek ölüm oranlarını azaltmak amacıyla, civcivlerde ovaryum transplantasyon işleminin kuluçkadan çıkışı takip eden 24. ya da 48. saatlerde uygulanması gerektiğini göstermektedir.

Anahtar sözcükler: Civciv, Ortotopik, Overektomi, Ovaryum, Transplantasyon



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INTRODUCTION

Preservation of the poultry germ plasm is of up-most importance as there is a steady decline in the number of poultry lines kept by the industry and public institutions¹. Therefore, there is a constant search to establish effective means to conserve the poultry germ plasm. Until recently, the only effective means to conserve the avian germ plasm remain to be keeping live animals. Cryopreservation of semen can only be used for single-gene traits and therefore falls short in preservation of the genetic variation². Although there are cell-based methods that can also be applied to this end³, they remain to be ineffective. A recent study by Song and Silversides⁴ suggested that orthotopic ovarian transplantation can provide an alternative solution to the problem provided that effective methods are developed for cryopreservation of the ovarian tissue. These authors established a surgical technique for orthotopic transplantation of the ovarian tissue for 1-day-old chicks, which appear to accept skin grafts and the ovarian tissue without any evidence of immune rejection. Further studies by the same authors culminated in the production of live offspring both in the chicken^{5,6} and the quail⁷. Taken together, these results strongly indicated that orthotopic ovarian transplantation in one-day-old chicks, when combined with cryopreservation of sperm, could indeed provide an effective means for the conservation of the avian germ plasm.

The orthotopic ovarian transplantation in newly-hatched chicks developed by Song and Silversides⁴ requires the removal of the yolk sac, the removal of the ovary of the host, and finally engraftment of the ovarian tissue pieces from a donor under general anaesthesia. Survivability of chicks after these rather complex surgical manipulations was reported to be 100%⁴. Experiments carried out in our laboratory, however, indicated that majority of newly-hatched chicks could not survive the surgical technique described by Song and Silversides⁴ and die within 24 h following the operation. A series of experiments were performed in the present study to determine whether or not it is the removal of the ovary or the removal of the yolk sac, a prerequisite for the ovariectomy operation, that is associated with the relatively higher mortality rates observed. Evidence gathered in the present study indicates that removal of the yolk sac has a major effect on the survival of operated chicks independent of the ovariectomy operation, and that this effect is strongly correlated to the timing of the operation following hatching.

MATERIAL and METHODS

Experimental Design

In the first series of experiments, a total of 50 newly hatched (day 0) White Leghorn chicks (Line W36, HyLine)

were ovariectomized in two replications using 25 chicks in each replication. Day 0 was defined as the day of hatching. Chicks having excessive haemorrhages during the operation were excluded from the study. Un-operated chicks (n=25) and newly hatched (day 0) chicks having abdominal incision only (n=25) served as controls for this series of experiments.

In the second series of experiments, the effect of the removal of the yolk sac on various time points in reference to the day of hatching was examined. To this end, the yolk sac was removed on day 0 (n=10, day of hatching), day 1 (n=11, 24 h post-hatch) and day 2 (n=8, 48 h post-hatch). In this second series of experiments, only the yolk sac was removed and the ovary was left intact.

Removal of the Yolk Sac and the Ovariectomy Operation

The surgical transplantation technique was approved by the Institutional Animal Ethics Committee (ADÜ-HADYEK, 2007/013). All operations were performed in a laminar cabin sterilized by exposure to UV light 1 hour prior to the operation using sterile surgical equipment to maintain asepsis and antisepsis throughout the operations. Newly hatched chicks were anesthetized by intramuscular injection of 0.5 mg of ketamin (Alfamine, Alfasan International B.V.) and 0.1 mg of xylazine (Alfazyne, Alfasan International B.V.). The abdominal skin was swabbed with 70% ethanol, and the feathers were removed from the area of operation. A 2.5-3 cm transverse incision was made approximately 1 cm distal to the last rib to expose the left side of the abdominal cavity. The yolk sac was removed after tying the connecting stalk with surgical suture and ovariectomy was performed as described by Song and Silversides⁴ during which careful attention was paid to avoid any excessive haemorrhages. Chicks having massive haemorrhage during the operation were culled and were not included in determining the survival/mortality rates. In the second series of experiments aimed to examine the effect of the removal of the yolk sac on the survival of newly-hatched chicks, only the yolk sac was removed leaving the ovary intact. The abdominal incision was closed by surgical suture and the operated chicks in all groups were administered 5 mg of an antibiotic (Baytril, Bayer Health Care) immediately after surgery. All operated chicks were kept on a heating block until recovery from the anaesthesia and then placed in a brooding machine.

Statistical Analysis

Differences in the number of chicks surviving the ovariectomy operation and the effect of the removal of the yolk sac on the survival of operated chicks in relation to the timing of the operation following hatching were analyzed using the Chi-square test. The statistical package SPSS (Version 11) was used for all analyses. A value of $P < 0.05$ was considered to be significantly different.

RESULTS

A total of 50 newly-hatched chicks (day 0) went through the ovariectomy operation in the first series of experiments. It was determined that the survival rate did not exceed 36% (Table 1) and majority of chicks died within 24 h following the operation. On the other hand, all of the un-operated chicks and chicks having an abdominal incision only did survive the operation (data not shown).

We considered in the second series of experiments the possibility that the removal of the yolk sac by itself would have an effect on the mortality rate as the yolk sac plays a crucial role as a nutrient reserve for the young hatchling⁸. In order to test this hypothesis, only the yolk sac of a group of chicks (n=10) was removed on the day of hatching (day 0) leaving the ovary intact. It was determined that only 30 % of these chicks could survive the operation and majority of the chicks died within 24 h following the operation (Table 2). When the yolk sac is removed on day 1 (n=11, 24 h post-hatch) only 27% of chicks died following the operation (Table 2). On the other hand, the removal of the yolk sac did not cause any mortalities in chicks operated on day 2 (n=8, 48 h post-hatch), 48 hours following the day of hatching.

to preserve the avian germ plasm. However, previous attempts for the transplantation of the ovarian tissue were not successful¹¹⁻¹³. On the other hand, a recent study performed by Song and Silversides⁴ indicated that, when the technique is applied to 1 day-old chicks, the immune system of the recipient chick tolerates the grafted tissue. A surgical technique established for orthotopic transplantation of ovarian tissue in newly hatched chicks was employed in subsequent studies demonstrating the feasibility of the approach for the production of live offspring both in the chicken^{5,6} and the quail⁷.

The orthotopic ovarian transplantation in newly-hatched chicks is a technically demanding procedure and involves the removal of the yolk sac, the removal of the ovary of the host, and finally engraftment of the ovarian tissue pieces from a donor under general anaesthesia. It has been reported that almost all of the operated chicks survive these rather complex surgical manipulations⁴⁻⁶. In contradiction to these observations, preliminary experiments carried out in our laboratory indicated that majority of newly-hatched chicks could not survive the surgical technique described by Song and Silversides⁴. In an attempt to determine the underlying causes of this unexpectedly high mortality rate associated with the

Table 1. Survival of chicks ovariectomized on the day of hatching (day 0)

Tablo 1. Kuluçkadan çıkış günü (0. gün) overektomi uygulanan civcivlerin hayatta kalma oranları

Replication No	Number of Operated Chicks		Number and Percentage of Live Chicks		Number and Percentage of Death Chicks	
	n	%	n	%	n	%
1	25	100	9	36	16	64
2	25	100	4	16	21	84

$\chi^2: 2.599, P=0.107$

Table 2. The effect of the removal of the yolk sac on the survival of chicks in reference to the day of hatching.

Tablo 2. Kuluçkadan çıkış günü referans alınarak yumurta sarı kesesinin uzaklaştırılmasının ölüm oranları üzerine etkisi

Post-hatching Time (Days) *	Number of Operated Chicks		Number and Percentage of Live Chicks		Number and Percentage of Death Chicks	
	n	%	n	%	n	%
0	10	100	3	30	7	70
1	11	100	8	73	3	27
2	8	100	8	100	0	0

* Day 0 was defined as the day of hatching, $\chi^2: 10.047, P=0.007$

DISCUSSION

Orthotopic ovarian transplantation is a powerful technique that can be put into use for maintaining lines of transgenic mice⁹ and is an active area of research aimed to preserve the fertility of young women diagnosed with cancer¹⁰. Similarly, orthotopic transplantation of cry-preserved ovarian tissue would facilitate efforts aimed

ovarian transplantation procedure, a series of experiments were performed in the present study.

In the first series of experiments, a total of 50 newly-hatched chicks went through the ovariectomy operation on the day of hatching (Day 0). It was determined that majority of chicks could not survive the surgical manipulations and died within 24 h following the operation. On the other hand, all of the un-operated chicks and chicks having

an abdominal incision only did survive the operation indicating that ineffective asepsis and antisepsis were not the primary cause of the deaths. Excessive bleeding during the operation, as low as 20% depending on the skill of the surgeon, was reported as the main reason for the failure of the transplantation technique⁴. However, this possibility is excluded in the present study as chicks having massive haemorrhage during the operation were culled and were not included in determining the survival/mortality rates.

Having established that ovariectomy operation on the day of hatching is associated with 64-84% mortality rate, the possibility that the removal of the yolk sac by itself would have an effect on the mortality rate was considered as the yolk sac plays a crucial role as a nutrient reserve for the young hatchling⁸. In order to test this hypothesis, only the yolk sac of a group of chicks (n=10) was removed on the day of hatching (day 0) leaving the ovary intact. It was determined that only 30% of these chicks could survive the operation and majority of the chicks died within 24 h following the operation (Table 2). It is interesting to note that the mortality rate determined in this experiment is close to that determined for the ovariectomized newly-hatched chicks (Table 1). It appears on the basis of these observations that it is the removal of the yolk sac that determines to a large extent the survival of chicks operated on the day of hatching even when the ovary remains intact.

It is well established that the yolk sac is absorbed by the newly-hatched chick and provides nourishment in the first few days following hatching. In close agreement to previous observations on the utilization of the yolk sac by the newly hatched chick¹⁴, the average weight of the yolk sac for chicks used in the present study was 5.7 g, corresponding to about 13% of the chick weight on the day of hatching (data not shown). More than half of the yolk is absorbed in the following 48 h such that the yolk sac weighs 3.2 g on average, corresponding to about 8% of the chick weight (data not shown) on day 2. We therefore considered the possibility that the timing of the yolk sac removal would also alter the survival of the operated chicks and that chicks would tolerate the operation better in subsequent days following the day of hatching. To test this hypothesis, the yolk sac was removed on day 1 (n=11, 24 h post-hatch) and on day 2 (n=8, 48 h post-hatch), day 0 being defined as the day of hatching. It was determined that only 27% of chicks died following the operation on day 1 in stark contrast to the mortality rate obtained in chicks operated on day 0 (Table 2). On the other hand, the removal of the yolk sac did not cause any mortalities in chicks operated on day 2, 48 h following the day of hatching. These data would strongly indicate that not only the removal of the yolk sac, but also the timing of this operation have a major effect on the survival of chicks going through the ovariectomy operation.

Taken together, evidence gathered in the present study indicates that majority of newly hatched chicks do not

survive the ovariectomy operation, a prerequisite for the orthotopic ovarian transplantation technique described recently by Song and Silversides⁴. Most importantly, it appears that it is the removal of the yolk sac on the day of hatching leading to a very high (64-84%) mortality rate in chicks whose ovary remained intact. These results are in contradiction to almost zero mortality rate described by Song and Silversides⁴. The discrepancy between these observations is most likely to be due to the timing of the operation as the removal of the yolk sac one or two days following the day of hatching (day 0) is associated with significantly less mortality rate. It would appear on the basis of these observations that the orthotopic transplantation of the ovarian tissue in the chick should be performed one or two days following the day of hatching in order to avoid a high mortality rate associated with the surgical technique. Whether or not performing the operation 24 or 48 h following the day of hatching alters the immune response of the recipient chick to the grafted tissue warrants further investigations.

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REFERENCES

1. Hagedorn M: Avian genetic resource banking: Can fish embryos yield any clues for bird embryos? *Poult Sci*, 85, 251-254, 2006.
2. Blesbois E, Seigneurin F, Grasseau I, Limouzin C, Besnard J, Gourichon D, Coquerelle G, Rault P, Tixier-Boichard M: Semen cryopreservation for ex situ management of genetic diversity in chicken: Creation of the French avian cryobank. *Poult Sci*, 86, 555-564, 2007.
3. Petite JN: Avian germplasm preservation: Embryonic stem cells or primordial germ cells? *Poult Sci*, 85, 237-242, 2006.
4. Song Y, Silversides FG: The technique of orthotopic ovarian transplantation in the chicken. *Poult Sci*, 85, 1104-1106, 2006.
5. Song Y, Silversides FG: Offspring produced from orthotopic transplantation of chicken ovaries. *Poult Sci*, 86, 107-111, 2007.
6. Song Y, Silversides FG: Long-term production of donor-derived offspring from chicken ovarian transplants. *Poult Sci*, 87, 1818-1822, 2008.
7. Song Y, Silversides FG: Transplantation of ovaries in Japanese quail (*Coturnix japonica*). *Anim Reprod Sci*, 105, 430-437, 2008.
8. Reidy TR, Atkinson JL, Leeson S: Size and components of poult yolk sacs. *Poult Sci*, 77, 639-643, 1998.
9. Szein J, Sweet H, Farley J, Mobraaten L: Cryopreservation and orthotopic transplantation of mouse ovaries: New approach in gamete banking. *Biol Reprod*, 58, 1071-1074, 1998.
10. Bedaiwy MA, Shahin AY, Falcone T: Reproductive organ transplantation: Advances and controversies. *Fertil Steril*, 90, 2031-2055, 2008.
11. Guthrie CC: Further results of transplantation of ovaries in chickens. *J Exp Zool*, 5, 563-576, 1908.
12. Davenport CB: The transplantation of ovaries in chickens. *J Morphol*, 22, 111-122, 1911.
13. Grossman M, Siegel PB: Orthotopic ovarian transplants in chickens. *Poult Sci*, 45, 1434-1436, 1966.
14. Deeming DC: Yolk sac, body dimensions and hatching quality of ducklings, chicks and poults. *Br Poult Sci*, 46, 560-564, 2005.