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# Searching of Pregnancy Rate in Repeat Breeder Cows by Embryo Transfer Practices

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ABSTRACT

The aim of this study is to compare the pregnancy rates by applying embryo transfer to the cows which are not pregnant and should be removed from herd as repeat breeder. In this study, 87 randomly selected Holstein cows utilized. The repeat breeder cows (n=45) were selected from at least one giving birth, having regular sexual cycle, missing clinical worsening into genital organ and not displaying an abnormal discharge. On the other hand, it was selected from non-pregnant cows which inseminated artificially at least 3 times or more. Besides, cows that used as the control group (n=42) were selected from the cows without any artificial insemination postnatally. The PGF2α application was performed to all recipient cows which are considered to benefit from as a recipient in control and testing groups just 24 days before the flushing day. After this application, the cows showing estrous symptoms were recorded and determined as candidate recipients. The pregnancy rates were 35.6% and 50% for testing and control groups, respectively and the difference between the groups was significant (P<0,05). As a result, even this difference between the groups it has been concluded that embryo transfer can be used to conceive especially for high-yielding cows as a treatment method for repeat breeder cows. Thereby, embryo transfer from the cows with high superior characteristics to the high milk yielding cows which have infertility problems can economically be beneficial by utilizing the high milk yield in the later lactation without any replacement cost.

Keywords: Embryo transfer, Holstein, Infertility, Repeat breeder

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## 1. Introduction

Many problems are faced affecting of cows milk and reproduction yield in countries where modern dairy farming is carried out. One of these is repeat breeder cow (RBC) problem. RBC is defined as cows that do not become pregnant even though they are inseminated at least 3 or more times. In a wide definition about this problem; the animals which is younger than 10 years old and had at least one birth, having regular sexual cycles, have not a clinical disorder in the genital organs and not showing an abnormal discharge, however, do not conceive of mating with a fertile bull or artificial inseminating 3 or more times are called repeat breeder cows (Alaçam 2010; Kaymaz 2012).

The ethiology of RBC syndrome is not fully know and has multifactoria causes. The cow, the bull and several environmental/handling factors are incriminated. All of them are often overlapped and it is difficult to determine the primary origin. While influence of the age of the mother, genetic factors, uterine infection and repeat estrous cycles, anatomical defects of the genital tract, hormonal dysfunctions, early embryonic deaths, inadequate follicular growth and effect of nutrition (the importance of nutrition in all vital processes is indisputable, and the qualitative and quantitative differences in the ration in dairy cattle may cause reproductive dysfunctions) can be considered as maternal reasons; influence of bull fertility and semen quality, site of semen deposition and estrus return, time of semen deposition are bull related factors. Except those, environmental factors can also be included in the ethiology of repeat breeder syndrome. These are season, stress, estrus detection, hygiene at artificial insemination and parturition (Perez-Marin et al. 2012).

Embryo transfer applications are one of the most important ways to assure genetic improvement rapidly in dairy cattle and increase the number of elite female and male animals in the herd (Seidel & Seidel 1991; Akyol 2001; Bilby 2010; Tekeli 2010). Besides, embryo transfer is one of the modern techniques used to increase success in animal breeding in the most effective way (Bülbül & Dursun 2005). The most important goal of embryo transfer applications consisting of a series of biological processes is to provide healthy and having good genetic capacity offsprings by obtained high quality oocytes and embryos (Santos et al. 2004).

Embryo transfer into lactating dairy cattle has shown beneficial effects in improving fertility in dairy cattle, especially during summer heat stress. The transfer of an embryo could bypass certain causes of infertility (i.e., fertilization failure and

early embryonic loss). Therefore, embryo transfer can be utilized to improve pregnancy rates in repeat breeder dairy cattle (Bilby 2010).

The aim of this study is to investigate whether it is possible to conceive by applying embryo transfer to the cows which do not conceive and be required to be selected from the herd despite being inseminated at least 3 times. Thus, it is tried that cows with RBC syndrome are conceived by embryo transfer and it will be shown that economic gain can be achieved by keeping them in herd for a while. Various studies have been worked in Turkey with repeat breeder cows. Most of these studies are based on artificial insemination with hormonal treatments. But, in this study, Repeat breeder cows are tried to be treated by fresh embryo transfer.

## 2. Material and Methods

### *2.1. Animals*

In this study, embryos obtained from lactating cows selected as donors at the Eastern Mediterranean Agricultural Research Institute were transferred to the cows that were trial group that was repeat breeder cows and the control group that did not receive any postpartum treatment. The cows in this enterprise are Holstein and their ages are between 3 and 8 years old. In the study, 87 head cows randomly selected. Repeat breeder cows (n= 45) determined from the cows which have given at least one birth, have regular sexual cycles, have no clinical problem in their genital organs and show no abnormal discharge, but are not pregnant even if artificial insemination has been performed three or more times. The cows to form the control group (n= 42) were selected from animals that were not inseminated after birth.

In the farm, cows are housed in open barns and fans are used with water spraying as cooling systems to minimize the heat stress, which is an important effect of the Mediterranean climate. Wheat straw, alfalfa, corn silage and vetch as roughage and concentrated feed with 18% protein produced in the enterprise was used in animals ration. Water was provided with automatic waterers as adlibitum.

### *2.2. Superovulation protocol and evaluation of embryos*

Progesterone release intravaginal device (PRID) (PRID-Delta, Ceva, Turkey) that releases controlled hormone (progesterone), the most preferred method to synchronize follicular development, were administered before superovulation protocol. Following this application, 7 days later, FSH (Folltropın-V, Bioniche Animal Health Europe Ltd., Ireland) injections were started to administere in decreasing doses intramuscularly (i.m.), 4 days-12 hours apart-twice a day-8 times in total. 2 cc PGF2α (Lutelen, Topkim, Turkey) injected on the mornings 3$^{rd}$ and 4$^{th}$ days of FSH application in order to regression of the existing corpus luteum and providing ovulation. PRIDs were taken out on the 3$^{rd}$ day evening of FSH injections. The donor cows were inseminated 3 times at the 12$^{th}$, 24$^{th}$ and 48$^{th}$ hours following the last FSH application. 2.5 cc GnRH (Receptal, MSD Animal Health, Germany) was injected with 2$^{nd}$ and 3$^{rd}$ artificial inseminations. Uterine flushing was made on the 7$^{th}$ day following the first insemination. Uterine flushing was performed by non-surgical method. Embryos were recovered with flushing medium in a sterile bottle. After the uterine flushing process is completed, 2 cc PGF2α was made to be lysed corpus luteum and 500 mg benzathine cefapirin (Metricure, MSD Animal Health, The Netherlands) was administered intrauterinely to prevent uterine infections. Embryo scanning were carried out in petri dishes using by heated stereo microscopes (Leica, S8APO, Japan) after flushing medium in sterile bottle was filtered and portioned out in smaller amounts. The recovered embryos were put in to culture solution (same as holding solution) and kept in an incubator (Binder, USA) providing 38.5 °C, 5% CO2 and high humidity environment until transfer.

Classification of recovered embryos for quality and development stages were made according to Kanagawa et al. (1995), Silva et al. (2009), and Kaymaz (2012)'s evaluation criterias.

### *2.3. Selection and preparation of recipient cows*

Repeat breeder cows selected as Trial Group (n=45) were determined from non-pregnant cows that they showed regular sexual cycles, no disorder was detected in their genital organs by clinical examinations although they were inseminated at least 3 times. The animals in the Control Group (n=42) were selected from the between cows that had at least 90 postpartum days, were showing a regular sexual cycle, had no clinical problems on their genital organs and had no artificial insemination.

A double dose of PGF2α injection, one of the methods of synchronization, was made to recipient cows in order to perform embryo transfer on the 7$^{th}$ day of oestrus. For this, the first PGF2α application was injected 24 days before the uterus flushing day. After 14 days, the second injection of PGF2α was applied and cows in oestrus were followed between 48$^{th}$ and 96$^{th}$ hours. Animals showing signs of oestrus were recorded and identified as candidates as recipients. Candidate cows were evaluated in both groups after ultrasound (Ultrasonic scanner, HS-101V, Honda, JAPAN) examination. Their uterus and ovarian structures were evaluated. Cows suitable to be recipient were selected for study. The fresh embryos were transferred to the recipient cows that had the appropriate corpus luteum (CL) on the day of the transfer and showed no pathological symptoms.

*2.4. Transfer of embryos*

The animals in the trial and control groups to be used as recipients should show oestrus 6-8 days ago. For this purpose, PGF2α injected intramuscularly twice with an interval of 14 days to cows that can be included in the study. These cows were monitored and those that showed oestrus symptoms were determined and recorded. The presence of quality CL in these determined cows recorded by using transrectal ultrasound technique; fresh embryo transfers were performed to the recipients which have good quality CL. Embryos of 7-8 days old obtained from donor cows were transferred by embryo transfer catheter to recipients' cornu uteri on the right or left where CL is detected by ultrasound. Pregnancy examinations in recipient animals were performed 28 days after transfer (35 days after oestrus) with ultrasound.

*2.5. Statistical analyses*

SPSS (Version 23) package program was utilized for statistical analysis of the study. Pregnancy of the groups was compared statistically using Chi-Square test.

## 3. Results and Discussion

Sixteen head repeat breeder cows were pregnant from 45 recipient cows in the trial group. In the control group, twenty one of 42 recipient cows which embryo were transferred, were diagnosed in pregnancy. As a result of the statistical analysis, pregnancy rates in the trial and control groups were 35.6% and 50%, respectively. In statistical comparison of both groups, there was a significant statistical difference between the rates of pregnancy at $P<0.05$ level ($p= 0.003$). Nevertheless, Considering the difficulties of getting pregnant with artificial insemination in repeat breeder cows, it can be said that the rate of pregnancy rate is high by performing embryo transfer. Pregnancy rates of the groups were found as shown in Table 1.

**Table 1- Pregnancy rates of the groups**

| *Groups* | *n* | *Pregnant* | *Non-Pregnant* | *Pregnancy %* | *Non-Pregnancy %* |
|---|---|---|---|---|---|
| **Trial Group** | 45 | 16 | 29 | 35.6 | 64.4 |
| **Control Group** | 42 | 21 | 21 | 50 | 50 |

Embryo transfer studies with repeat breeder cows are few. While other studies mostly were performed with frozen-thawed embryos, fresh transfer process was carried out in this study.

The pregnancy rates following either a controlled internal drug release (CIDR)-based timed artificial insemination (TAI) or an embryo transfer (TET) protocol were compared in lactating repeat breeder dairy cows. In the pregnancy examination performed on the 60th day, the rate of conception by embryo transfer was higher in repeat breeder cows compared to artificial insemination. It has been reported that embryo transfer is effectively increase the pregnancy rate in lactating repeat breeder dairy cows (Santos et al. 2004).

Dochi et al. (2008), thawed the embryos they obtained with in vitro fertilization application after freezing and transferred them to repeat breeder cows. Embryos were transferred 7 or 8 days after a natural estrus into one of two groups of animals that were either inseminated with frozen-thawed semen or were not inseminated (without-AI group) in the same estrous cycle as the embryo transfer. Both heifers and cows had higher pregnancy rates following embryo transfer and AI (with-AI group) than following embryo transfer only (without-AI group) (49.2% vs 29.5%; 41.5% vs 20.4%). Compared to this study and Dochi et al.'s study (2008), we had a higher rate of pregnancy rate in the group of directly embryo transferred. This may have been caused by Dochi et al. (2008) using frozen thawed embryos. Because pregnancy rate is lower in frozen embryo transfer compared to fresh transfer (Riha et al. 2002; Kızıl et al. 2012). In a study of non-pregnant cows despite artificial insemination at least 3 times, PGF2α treatment was performed to show the cows oestrus and it was found that the rate of conception was high as a result of embryo transfer in recipient cows (Rodrigues et al. 2010).

Stradaioli et al. (2015) have worked with 44 referred as clinically normal repeat breeder cows of 6 different dairy herds. Pregnancy rate after embryo transfer performed to repeat breeder cows was found 37.14%. In 6 dairy farms they have chosen for the study, it has been reported that pregnancy rates after artificial insemination vary between 20-35%. In both studies revealed that, further studies need to be made and embryo transfer may be a good treatment alternative in repeat breeder cows.

Karasahin et al. (2016) transferred to repeat breeder cows after the embryos are frozen by vitrification method. On the 60$^{th}$ day after the transfer, pregnancy examination was performed by rectal palpation and the pregnancy rate was found 28.13%. Comparing the study presented with this study, it is seen that the pregnancy rate is low in this study, although the results are close. The fact that frozen embryos were used in the study of Karasahin et al. (2016) and pregnancy examinations were performed on the 60th day may be the reasons for the low pregnancy rate compared to our study. It should also be considered that late embryonic deaths may have reduced the pregnancy rate. Late embryonic death-fetal death rates have been reported as

14%, 7.2% and 6.9% in different studies (Aslan & Wesenauer 1999; Silke et al. 2002; Kızıl 2011). Santos et al. (2004) reported the rate of embryonic death at the age of 30 to 45 days as 0.23% to 2.67% (average 0.85%) and overall 3.2% to 42.7% (average 12.8%).

In the study, the pregnancy rate in the repeat breeder cows group was found to be 35.6%. Whereas, it is very difficult to conceive these animals, which are defined as repeat breeder cows, by artificial insemination. There are studies reporting pregnancy rates as 20% and 18.75% in repeat breeder cows when artificial insemination is performed without applying any treatment (Selvaraju & Veerapandian 2010; Ergene 2012). In this case, it shows us that embryo transfer application is a useful and suitable alternative for the treatment of this syndrome by getting pregnant repeat breeder cows.

Three individuals may be involved in reduce pregnancy rates in natural service or even AI. These are sire, dam and embryo. Pregnancy rate generally depends upon three individuals who interact in various ways, often in a common environment. Heat stress at day 1 or days 1 - 3 reduced embryo survival and pregnancy rates. However, heat stress at days 3, 5 or 7 did not affect pregnancy rates indicating that thermal tolerance is acquired as the embryo ages. Considering that there is no sperm utilizing for recipient cows due to embryo transfer and the embryo to be transferred to the recipient is good quality and has passed a specific stage (age), by eliminating the other two factors, only the factor that may arise from the recipient cow remain. To put it more clearly, this allows us to eliminate two important disadvantages that reduce the rate of conception and also, Since the embryo to be transferred will be at the age of 7 days, high results can be obtained compared to artificial insemination in the rate of conception under heat stress. It is stated that it is possible to achieve high pregnancy rates with embryo transfer by using frozen-thawed embryos in seasons when hot stress is effective and causes infertility (Rutledge 2001).

## 4. Conclusions

Consequently, according to the data obtained, ıt has been concluded that embryo transfer applications can be recommended as a successful treatment alternative for repeat breeder cows and the determination of the presence of corpus luteum on the transfer day with synchronous heat detection in the recipient cows to be transferred is of great importance in terms of pregnancy rates to be achieved. So, it can be helped to the breeding of the herd or animal with the transfer of the embryos of the cows with superior characteristics to the cows with superior characteristics but having fertility problems and also more efficient use of repeat breeder recipients in production. Embryo transfer is one of the most important and effective methods used in animal breeding with new methods and technologies developed in the last 50 years. It is also evaluated that with the introduction of widespread use in our country, it will make important contributions to meet our quality breeding pregnant heifer needs with more affordable costs.

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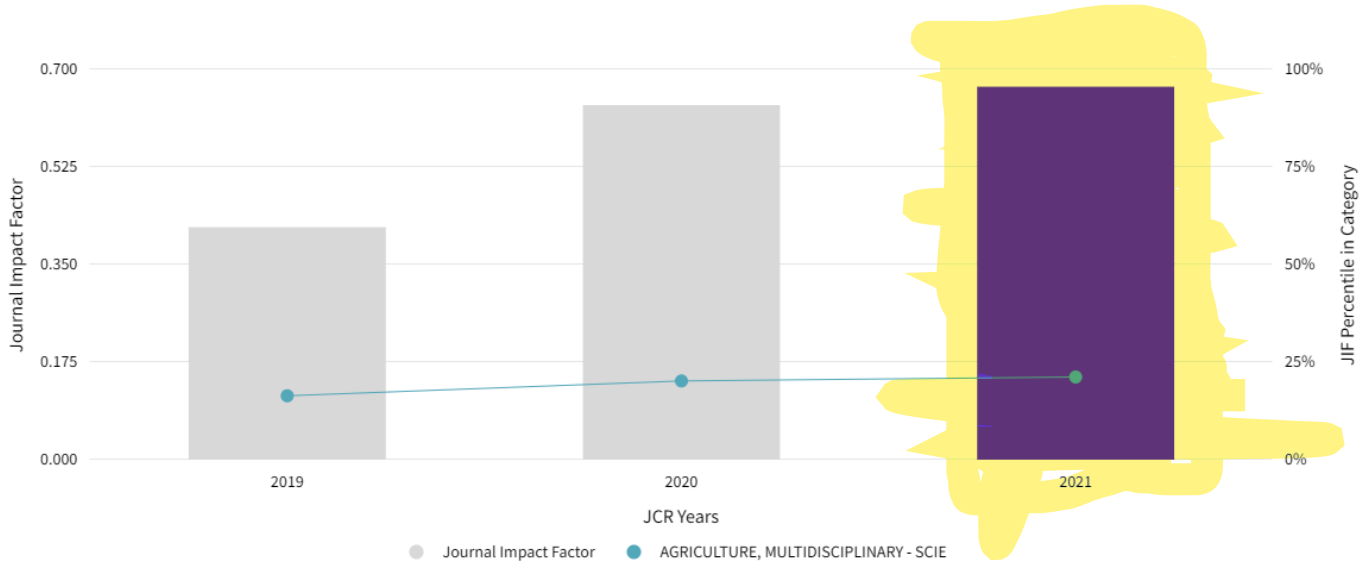
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