International Journal of Animal Science and Technology
2024; Vol. 8, No. 2, pp. 14-20
https://doi.org/10.11648/j.ijast.20240802.11

Research Article

Survival and Development of in Vivo Produced Boran and Boran* Holstein Cross Embryos

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Abstract

One of biotechnology technique that is frequently used to enhance the number of animals with superior genetic ability and high productivity is embryo transfer. Embryos can be obtained in vivo or in vitro, and they can be frozen and then thawed before being delivered to the recipient animals. Conception rates are influenced by a number of variables, including the quality and developmental stage of the embryo, the location of the embryo's deposit in the uterus, the degree of difficulty of the transfer, whether to use a fresh or frozen embryo, the operator's experience, the corpus luteum's quality, whether to use a heifer or a cow, and the time of year the transfer occurs. The fertility of domestic animals is severely impacted by early embryonic death. For this reason, this study was carried out to gather data on early embryonic development that is normal, the amount and timing of embryonic mortality, potential endogenous and exogenous causes of embryonic loss, and to develop strategies to lower embryonic mortality. A total of 40 embryos (20 fresh and 20 frozen, 26 quality grade 1 and 14 quality grade 2, 29 compact Morula stage and 11 early Blastocyst stage) were transferred to 40 recipient cows (22 Boran and 18 H-B cross) with different body condition score. Return to heat was used as method of pregnancy diagnosis and all recipients were followed around day 14 post embryo transfer and 14 animals were shown heat sign, the rest 26 animals were suspected for pregnancy (65%). Using ultrasound, a pregnancy diagnosis was made on day 45, and 20 recipient animals were found to be 50% positive for PD. On day 60, PD positive animals were re-examined with ultrasound and only 10 were confirmed as PD positive (25%). The PD negative on day 45 and day 60 were suspected to be early embryonic mortality. Other pregnancy loss occurred in this study was abortion nearly after five month of pregnancy. It is not doubtful that, the technique of embryo transfer is utilized to increase the reproductive rates of important female animals. However, it needs proper management for both donor and recipient animals. Therefore, for the successful application of the technology optimum level of feeding both quantity and quality, health management and conducive environment should be fulfilled for all animals.

Keywords

Donor Animal, Embryos, Mortality, Recipient Animal

1. Introduction

Reproductive technologies are becoming a necessary tool to increase the efficiency of food animal production to meet the demands of the growing population. As the need for food increases, producers rely on innovative strategies to capitalize

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Received: 12 March 2024; **Accepted:** 29 March 2024; **Published:** 17 April 2024

on superior genetics and enhance fertility [1]. Utilizing embryo transfer, a popular biotechnological approach, is one such tactic to raise the population of animals with superior genetic ability and high productivity [2]. Cattle embryos are delivered to recipient animals either fresh or frozen and thawed. They can be obtained both in vivo and in vitro. A thorough investigation has been carried out to improve the effectiveness of these techniques, the rates of pregnancy, and to determine the factors influencing these rates [3-5].

After transferring embryos from donor animals to eligible recipients, the observed conception rate ranges from 30 to 60% [6, 7]. Numerous factors, including the embryo's developmental stage and quality, the location of the embryo's embryonic deposit in the uterus, the degree of difficulty of the transfer, whether to use a fresh or frozen embryo, the operator's experience, the corpus luteum's quality, whether to use a heifer or cow, and the time of year the transfer occurs, can influence low or high conception rates [3-8].

Fertility in domestic animals is severely impacted by early embryonic mortality. It has a significant role in the low reproductive efficiency of cows raised for dairy and beef. In cattle, especially lactating dairy cows, a significant percentage of embryonic loss happens in the first two to three weeks following conception, prior to the mother realizing she is pregnant, which happens around day sixteen [9]. The conceptus's delayed or inadequate production of IFN-tau, which results in a delay or failure to signal its presence to the mother for MPR, utero-embryonic asynchrony, and an early increase in endometrial PG F2 alpha (PGF2 alpha) secretion are all potential causes of embryonic loss during this crucial period. Furthermore, inadequate nutrition leads to low quantities of insulin-like growth factor and decreased progesterone, which disturb the uterine environment and increase the risk of fetal death [10]. Therefore, this study was conducted with following objectives: -

1) To generate Information on the amount and timing of embryonic death, the typical early stages of embryonic development, and potential endogenous and external causes of embryonic loss.
2) To determine factors influencing the rate of pregnancy in Boran cattle (*Bos indicus*) after embryo transfer.
3) To Draw strategies to reduce embryonic mortality.

2. Materials and Methods

2.1. Study Area

Study was carried at the Debre Zeit Agricultural Research Center (DZARC), which is located roughly 45 kilometers east of Addis Abeba. The latitude and longitude are 08 °44 N and 38 °58' E, 1900 meters above sea level. The long-term average annual rainfall is 851 mm, and the average annual temperature is 8.9 ℃ min & 28.3 ℃ max (mean = 19 ℃) (from Agro-meteorology 2018). Established more than 50 years ago on 130 hectares of land, DZARC is home to approximately 100 dairy animals, 52 of which are Boran breeds and the remaining 48 of which are cross-bred. The main reasons the animals are kept alive are for scientific research purposes related to nutrition, diseases, reproduction, and breeding. The dairy farm uses a free stall system with zero grazing, allowing the animals to run around in the yard. There is a separate laboratory for nutrition, animal health, and feed analysis in addition to one reproducible biotechnology lab. Dairy cattle Genetic improvement, breed conservation and improved breed multiplication with the help of Assisted reproductive biotechnology were the main focus of research program.

2.2. Experimental Animal

A total of 8 Donor cows were included in this study following a clinical and gynecological examination of the reproductive systems for any anomalies, and a use of rectal palpation and ultrasonography to assess each animal's reproductive status. For super ovulatory treatment, only cycling (with active CL) and seemingly healthy donor cows free of any reproductive disorders were chosen as candidates.

The housing, feeding, and health care of all the cows were the same: they were given green fodder (fresh grass, alfalfa, and elephant grass) and commercially prepared concentrate feed (mixed from wheat bran, wheat middling, corn, Noug cakes, and mineral salts) in addition to teff straw and grass hay as a basal diet. Water was provided daily ad libitum, and feeding was determined by the animal's stage of reproduction and degree of production. Every year, the animals received vaccinations against anthrax, black leg, FMD, pasteurellosis, CBPP, and LSD in addition to being dewormed with a broad-spectrum anthelmintic.

In this investigation, ethical considerations were given careful thought. The experimental animals are not subjected to any unnecessary stress throughout any of the processes, and because animal handling is done humanely, the animals have not suffered injury or needless suffering.

2.3. Donor Super Ovulatory Procedure

The reproductive organs of all selected donor cows were assessed by rectal palpation and real-time B-mode ultrasound using a 5 MHz linear array probe (SIUI, Altay Scientific S.P.A., Italy) to determine the type of the cervix and the state of CL. Eight donor cows in all—four Boran and four Cross-breed—were employed in this study. Day 0: Both breeds received a seven-day CIDR (Proges-terone 1.38 gm, Hamilton, New Zealand) injection. The cows were divided into two groups on the fourth day of therapy, with 250 IU of FSH (Pluset®, Spain) for Boran and 700 IU for Cross. FSH was injected intramuscularly twice daily with a 12-hour gap between injections for four days in consecutive days, following a decreasing dose protocol (Table 1). Day 6 saw the intramuscular injection of 5 ml of PGF2α (Lutalyse®, Spain), and Day 7 saw the withdrawal of CIDR during the final FSH injection.

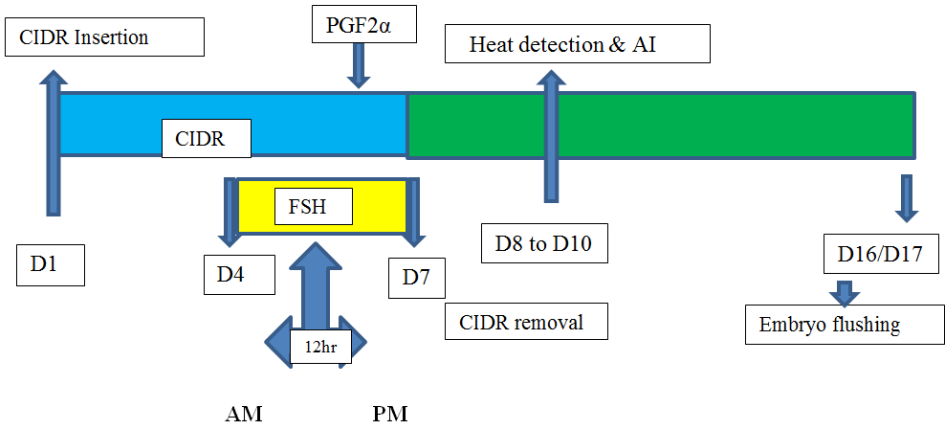

Figure 1. *Donor cow's superovulation and embryo recovery protocol [11].*

2.4. Heat Detection and AI of Donor Cows

Following the withdrawal of CIDR, heat signs were carefully monitored, and animals with brief oestrous periods were controlled with the use of a heat detector (ESTROTECTTM #U.S.pat. #6,467,430). In order to match the ovulation time with post-sperm capacitation, all cows were inseminated twice at a 12-hour interval following standing heat, based on the observed heat sign.

2.5. Response Evaluation and Embryo Collection

Super ovulatory response was measured on day 17 before to embryo flushing using a real-time B-mode ultrasonography with a 5 MHz linear array probe (SIUI, Altay Scientific S.P.A., Italy) by counting the total number of CL and unovulated follicles on the ovaries. Using a two-way Foley catheter (18 Fr 650 mm length; MOFA®, Canada) and 1000 cc of commercial flushing medium (ViGROTM, Bioniche Animal Health, USA), the embryos were harvested using a non-surgical method.

2.6. Evaluation and Grading of Embryos

The media inside an embryo filter was flushed, and then it was moved to a searching dish so that embryos and UFOs could be seen via a stereomicroscope (Motis SMZ 140/143®, Roanoke, USA). The harvested embryos, also known as UFOs, were placed into four well plates filled with holding medium. According to the International Embryo Transfer Society (IETS, 2013) manual, the embryos were graded according to their developmental stage (from stage 1 = one cell to stage 9 = enlarged hatched blastocyst) and quality (from quality 1 = good to quality 4 = degenerate). The obtained embryos were classified as transferable (those in 4–8 developmental stages and a quality grade of 1 & 2), degenerate, and UFO in accordance with the IETS guidelines.

2.7. Estrus Synchronization of Recipients

A total of 50 recipient cows (25 Boran and 25 Holstein Boran cross breed) were selected after a clinical and gynecological examination of the reproductive systems for any anomalies, and to use rectal palpation and ultrasonography to assess each animal's reproductive state. Only cycling and healthy cows were selected and they were overseen by comparable housing, food, and health management in parallel to Donor Cows management. CIDR (Progesterone 1.38 gm, Hamilton, New Zealand) were implanted on day 0 for all selected recipients for seven consecutive days and on Day six 5ml of PGF2α (Lutalyse®, Spain) was given IM. Finally, CIDR was withdrawn on Day 7 and (ESTROTECT^{TM} #U.S.pat. #6,467,430) was applied for heat detection.

2.8. Recipient Evaluation and Embryo Transfer

From both breed 50 treated animals only 40 animals (22 Boran and 18 HB cross) were responded for estrus synchronization treatment. Seven days after the candidate recipient cows were shown heat sign Transrectal ultrasonography was used to assess the ovaries in order to identify the presence, size, and location of corpora lutea (CL). Fresh or frozen embryos were randomly assigned to cows with active CL. After being taken out of the liquid nitrogen container, straws containing frozen embryos were submerged for 15 to 30 seconds in a water bath that was heated to 35 ℃. After that, the embryos were placed into the uterine horn ipsilateral to the CL using an embryo transfer gun (ET Gun 17274, WTA, College Station, TX, USA) filled with straw. Twenty fresh and twenty frozen embryos were transplanted in total.

2.9. Pregnancy Diagnosis

Pregnancy examination of recipients was undertaken by recording those animals coming back to heat at around 14days

after embryo transfer, utilizing a real-time B-mode ultrasonography on days 45 and 60 following embryo transfer with a 5 MHz linear array probe (SIUI, Altay Scientific S.P.A., Italy). During this investigation, it was hypothesized that the reason why animals were observed in heat sign after 14 days of embryo transfer was disruption of the embryo-maternal interactions, which results in embryonic loss when the mother is not aware that she is pregnant. Other animals were examined at day 45 with ultrasound and animals with dark black ultrasound images were recorded as pregnancy positive. On day 60 again the pregnancy examination was performed with ultrasound to observe the heartbeat of the fetus and pregnancy loss was recorded as there was no fetal heartbeat observed as well as the dark fetal fluid observed during day 45 examination was reabsorbed.

Data analysis: Data on body condition score, Recipient response rate to synchronization treatment, embryo quality, abortion, calf born and embryonic loss at different level was recorded to Microsoft excel and summarized using descriptive statistics.

3. Results

3.1. Recipient Response to Synchronization Treatment

All recipient animals were selected based on their history of healthy status, non-lactating and with recommended reproductive status. Accordingly, animals with range of Body condition from 2.75 to 3.5 from both breed was included in the experiment. A total of 50 recipient animals (equally from both breed) were received synchronization treatment and only 40 animals (22 Boran and 18 H-B cross) were responded to the treatment.

Table 1. *Synchronization of recipient animals and response evaluation.*

Animal Category	№ of treated animals	№ Responded animals	Response rate
Boran	25	22	88%
H-B cross	25	18	72%
Total	50	40	80%

3.2. Quality of Transferred Embryos and Pregnancy Establishment

A total of 40 embryos (20 fresh and 20 frozen, 26 quality grade 1 and 14 quality grade 2, 29 compact Morula stage and 11 early Blastocyst stage) were transferred to 40 recipient cows (22 Boran and 18 H-B cross) with different body condition score. Return to heat was used as method of pregnancy diagnosis and all recipients were followed around day 14 post embryo transfer and 14 animals were shown heat sign, the rest 26 animals were suspected for pregnancy (65%). On day 45 pregnancy diagnosis was undertaken using Ultrasound and 20 recipient animals were recorded as PD positive (50%). On day 60, PD positive animals were re-examined with ultrasound and only 10 were confirmed as PD positive (25%). The PD negative on day 45 and day 60 were suspected to be early embryonic mortality.

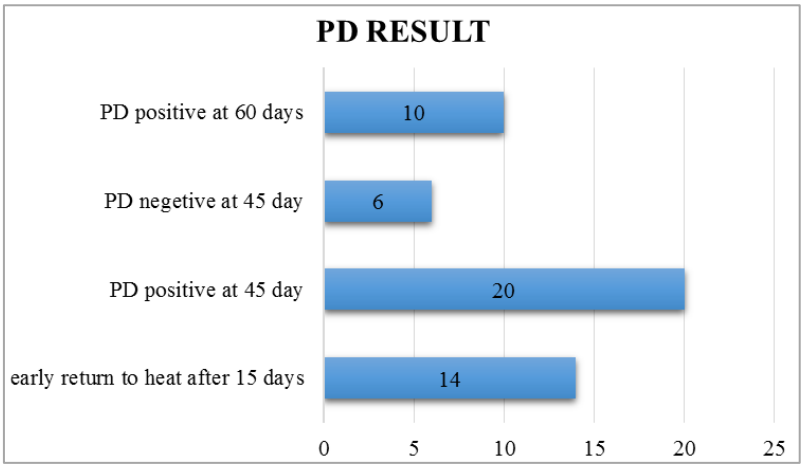

Figure 2. *Pregnancy diagnosis of recipient animals.*

Table 2. Quality grade of transferred embryos and pregnancy diagnosis result.

Animal category	№ of quality grade 1 embryos	№ of quality grade 2 embryos	early returned to heat after 15 days	№ PD positive at day 45
H-B cross	12	6	5	8
Boran	14	8	9	12
	26	14	14	20

3.3. Pregnancy Losses

The embryonic characteristics of the recipient and the transfer technique can be linked to deaths that occur between days 45 and 60 after embryo transfer [12]. In this study embryonic loss was occur at different stages, on day 14 post embryo transfer 14 animals were returned to heat and this was an early embryonic loss due to the disturbance of the embryo–maternal interactions in the event that the mother does not recognize the pregnancy. Pre day 45 PD examination 6 embryonic loss and post day 45 PD examination 10 embryonic loss were recorded which can be due to insufficient Progesterone hormone. Other pregnancy loss occurred in this study was abortion nearly after five month of pregnancy.

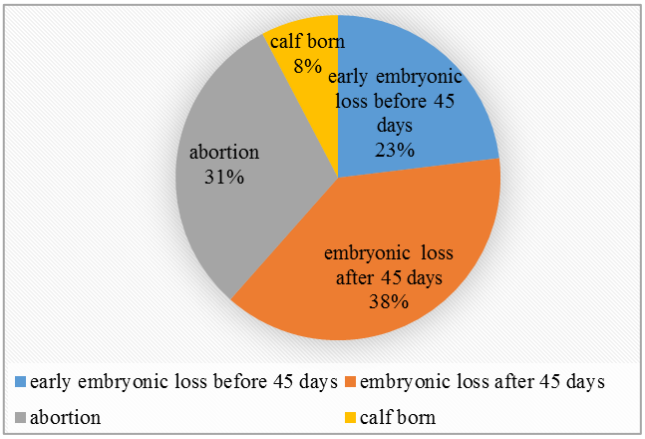

Figure 3. Embryonic loss recorded at different level.

4. Discussion

Embryo Transfer is the quickest method for altering genetic potential and permitting a rise in cow output. Because embryo transfer requires more money and takes longer than artificial insemination, it is crucial to raise pregnancy success rates. This study assessed the BCS and quality of transferred embryos, which were seen across the two different breeds of recipient animals, as parameters that influence the success rate of ET. Low pregnancy rates are associated with difficult cervical passage and prolonged embryo transfer times [8, 13, 14]. However, this study did not take into account other factors such the place of embryo deposition, embryo status, stage development, technician ability, level P4, or the transfer procedure.

The reproductive efficiency of dairy or beef cattle is affected by elements such as unsuccessful fertilization and embryonic death. Repeat breeding, which raises the expense of artificial insemination, lengthens calving intervals, prolongs the dry season, and reduces life-time milk output and calf production, is a primary cause of early embryonic mortality and economic loss [15]. In cattle, about 35% of embryonic-fetal deaths occur [16]. between days 7 and 16 in particular, the first three weeks following insemination account for 70 to 80 percent of the entire embryonic loss. Non-lactating cows have higher embryo mortality than heifers, and early pregnancy loss in nursing dairy calves can reach 70%. Another significant cost element in the cattle embryo transfer (ET) sector is infertility and subfertility. After in vivo-derived embryos from superovulated donors are transferred, the mean survival rate to calving is just 43%, with a range of 31 to 60%. [17] which is very align with this current study.

An average embryonic loss occurs between Days 8 and 27, coinciding with embryo elongation and the mother's awareness of her pregnancy. This loss is roughly 30%. Between Days 28 and 60, around 12% and approximately 2% pregnancy losses occur in third month of gestation [18]. In order to guarantee that enough IFNT is secreted and to increase the conceptus's surface area for the greatest possible circulatory exchange with the mother's tissues following implantation, elongation is required. It is believed that one of the main causes of cow reproductive failure is the conceptus's incapacity to elongate optimally, which inevitably leads to embryonic loss [19]. During this study 14 animals who received embryos were comeback to heat within the first estrous cycle which was due to the embryos was unable to produce enough concentrations of IFNT and no establishment of embryos in the event that the mother does not recognize the pregnancy.

A portion of embryonic death may also be related to nutritional effects on reproductive processes, which can be influenced by a variety of factors including body type, diet, and physiological state. These factors can have an impact on the reproductive system, acting either individually or in combi-

nation at the level of the hypothalamus/pituitary axis, the ovary, the oocyte, the embryo, and the uterus. Progesterone levels at their ideal range are crucial for fertility. Progesterone and embryonic IFN-τ have recently been shown to be positively connected, and slight variations in the mother's progesterone levels may vary the anti-luteolytic agent's release and impact the survival of the embryo. [20, 21].

The Low levels of P4 in the cycle before estrus have an impact on the survival of the resulting embryos by causing oocytes to mature too soon, which compromises their capacity to continue developing normally after fertilization [17]. The ovary's progesterone stimulates and sustains endometrial activities that are essential for the growth, implantation, placentation, and development of the fetus to term. P4 concentrations during early pregnancy in cattle have a discernible impact on embryonic survival. [22, 23]. Many study were concluded that the embryo quality can causes less pregnancy rates. During this study, quality grade 1 and 2 embryos were transferred. The pregnancy loss was occurring at different levels during this study, however the exact reason for pregnancy loss cannot identified.

Limitation of the study: during this study, the blood sample from all recipient animals were collected during embryo transfer on day 7, 14 and 21, serum separated and stored for later hormonal analysis (P4 and other hormone related with pregnancy). Due to lack of access to hormonal analyzer, it was not possible to process the samples.

5. Conclusion and Recommendation

It is not doubtful that, the technique of embryo transfer is used to increase the rates of reproduction of valuable female animals. However, it needs proper management for both donor and recipient animals. Therefore, for the successful application of the technology optimum level of feeding both quantity and quality, health management and conducive environment should be fulfilled for all animals.

Abbreviations

AI: Artificial Insemination
BCS: Body Condition Score
CBPP: Contagious Bovine Pleuropneumonia
CIDR: Controlled Internal Drug Release
CL: Corpus Luteum
DZARC: Debrezeit Agricultural Research Center
ET: Embryo Transfer
FMD: Foot and Mouth Disease
FSH: Follicle Stimulating Hormone
IFN-τ: Interferon Tau
IM: Intramuscular
LSD: Lumpy and Skin Disease
MPR: Maternal Pregnancy Recognition
PD: Pregnancy Diagnosis
PGF2ά: Prostaglandin F2 Alpha
P4: Progesterone
UFOs: Unfertilized Oocytes

Conflicts of Interest

The authors declare no conflicts of interest.

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