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Effects of Breed and Body Condition on Embryo Quality and Developmental Stage of *In vivo* Produced Embryos of Boran and Holstein-Boran Cross Breed

Mosisa Dire and Tamrat Degefa

Ethiopian Institute of Agricultural Research,
Debre Zeit Agricultural Research Center, P.O. Box: 32, Debre Zeit, Ethiopia

Abstract: Embryo transfer is one of the most important reproductive biotechnologies that used to multiply the number of animals with high genetic value and accelerates the improvement of animal genetic. This study was designed to evaluate the effects of breed and body condition on quality and developmental stages of *in vivo* produced bovine embryos. From 146 embryos, 60 embryos collected from Boran (41.1%) and 86 (58.9%) collected from Holstein Boran cross breed with, 83 (56.8%) embryos were transferable, 42 (28.8%) were unfertilized oocytes and 21 (14.4%) were degenerated embryos. From the total transferable embryos, 43 (51.8%) were of quality grade 1, 40 (48.2%) were of quality grade 2 and 58 (69.95%) were of morula stages and 25 (30.1%) blastocyst stage embryos were collected. In conclusion, there is no such breed effects on *in vivo* produced embryo quality as well as number of transferable embryos rather under BCS or over BCS (thin/fat) of donor cows affecting the quality of embryos, number of transferable embryos as well as overall embryo recovery rate. Therefore, if proper nutritional status can be maintained for donor cows on superovulation treatment by nutritional management, it is possible to enhance the utilization value of donor cows by increasing embryo recovery rate and the number of transferable embryos *in vivo* embryo production.

Key words: Body Condition · Breed · Donor Cow · Embryo Quality

INTRODUCTION

In the developing countries, the overtime increase in the number of populations has significantly increases the Demand for animal source products such as meat and milk [1]. The key to successfully meet the increasing demand for livestock products is to enhance productivity through improved genetic gain [2]. The desire to improve animal genetics can be achieved by using reproductive biotechnologies such as artificial insemination (AI) and multiple ovulation embryo transfer (MOET) [3, 4]. Generally, crossbreeding is considered as an effective tool for quick genetic improvement of low milk-producing cows [5].

Embryo transfer (ET) in cattle is one of the most important reproductive technologies after artificial insemination (AI). It is a tool of reproductive biotechnology that used to multiply the number of animals with high genetic value and accelerates the improvement of animal genetic [6, 7]. The purpose of ET is to increase the reproductive value of the cows as well as to enhance the productivity of the cows during their live. This is because of some cows have a low reproductive rate and having too long generation interval [8].

Several animal-related, environmental, technical and management factors affect the outcome of superovulation and embryo recovery. The efficiency of superovulation has remained relatively constant for over three decades [9]. The *in vivo* embryo quality after multiple super ovulations, artificial insemination (AI) and embryo recovery depends on the BCS of the donor cow [10]. The nutrient requirements (NR) affect nutrition intake, blood metabolites, ovarian function, the estrus cycle and fertility [11]. There is some evidence that breeds differ in responsiveness to superovulation treatments and capacity of producing higher number of transferable embryos [12].

Corresponding Author: Mosisa Dire, Ethiopian Institute of Agricultural Research, Debre Zeit Agricultural Research Center, P.O. Box: 32, Debre Zeit, Ethiopia.

Body condition can affect yield and quality of oocytes and embryo in different ways. Diet has been positively correlated with the growth rate and size of the ovulatory follicle [13, 14]. Yield and quality of oocytes and embryos upon superovulation reflects potential fertility of dairy cows. The success of ovarian response to superovulatory treatment is dependent on factors inherent to each individual animal, the breed being used, season of the year and nutritional status [15]. According to Siddiqui *et al*. [16], cows with moderate BCS (2.5-3) are likely to respond better to superovulation treatment than those with high BCS (4-5) because cows with higher BCS are more likely to acquire ovarian cysts and less ovulation. The livestock potential in Ethiopia is non profitable unless their genetic improved with assisted reproductive biotechnologies like artificial insemination, embryo transfer and others. Embryo transfer was started in Ethiopia before 30 years and suspended due to lack of input supply, equipment and skilled man power. However, recently the efforts as well as the results of some researchers were promising [17-19].

The nutrient requirement for animals affects their BCS as well as the physiology of their reproductive system. Specifically, the follicular development and superovulatory of donor cow is highly dependent on their body condition. Therefore, the objective of this study is to evaluate the effects of Breed and BCS of donor cows on *in vivo* produced bovine embryo quality and their developmental stages in Boran and H-B cross breed cows.

MATERIALS AND METHODS

Study Animals: The study was carried out for the last 10 months (March to December) on 24 donor cows (12 Boran and 12 Holstein Boran cross breeds) at Debre Zeit Agricultural Research Centre (DZARC). Cycling donor cows with 3 to 4 range of body condition score by 1 to 5 scale body condition scoring system were selected after clinical and gynaecological examination of the reproductive tracts for any abnormalities as well as to determine the reproductive status of the candidate animals. Selected donors were housed under similar facilities, fed complete rations and allowed free access to water.

Superovulatory Procedure: On day 0, all donor cows with active CL were implanted with CIDR (Progesterone 1.38 g, Hamilton, New Zealand) for seven days. On Day 4, cows were subjected to FSH (Pluset®, Spain) treatment which was administered through intramuscular injection for four consecutive days in a decreasing dose regimen and twice per day with 12 h interval(50, 50IU, 37.5, 37.5IU, 25, 25IU, 12.5, 12.5IU for Boran and 125, 125IU, 100, 100IU, 75, 75IU, 50, 50IU for Holstein Boran cross breed). On Day 6, 2ml of PGF2α (Estrumate®, France) was injected IM and CIDR was withdrawn on Day 7 at the last injection of FSH. Different dose of FSH was used for both breeds of donor cows (250 IU for Boran and 700 IU for crossbreds) [18, 19].

Insemination of Donor Cows: All superovulated donors were inseminated 12 hours after the onset of standing estrus and the second insemination was given after 8 hours. The same batch of frozen semen of similar source was used for all donors.

Embryo Collection, Evaluation and Grading: On day 7 after the last insemination (day 0), superovulatory response was evaluated by rectal palpation and real-time B-mode ultrasound with 5 MHz linear array probe (SIUI, Altay Scientific S.P.A., Italy) was used for counting the total number of CL and the unovulated follicles present on both ovaries. A 3 to 5 ml of epidural anaesthesia was injected before insertion of collection catheter. The embryos were collected using non-surgical [16] technique of embryo flushing with 1000ml of commercial flushing medium (ViGRO™, Bioniche Animal Health, USA). Collected embryos/UFOs were transferred to holding medium in four well dishes. The stereomicroscope (Motis SMZ 140/143®, Roanoke, USA) was used for embryo searching, evaluation and loading. The embryos were graded based on their developmental stage (from stage 1 = one cell to stage 9 = expanded hatched blastocyst) and quality (from quality 1 = excellent to quality 4 = degenerate) according to the manual of International Embryo Transfer Society.

Statistical Analysis: The data were analyzed using SPSS statistics 20.0 IBM Corp and P value was calculated using ANOVA to find any significant relationship. Statistical significance of the study considered P-value less than 0.05 as significant and P-value greater than 0.05 as non-significant.

RESULTS

In this study, a total of 146 embryos were collected. 60 embryos (41.1%) were collected from Boran and 86 (58.9%) were obtained their crosses yielded 83 (56.8%) transferable, 42 (28.8%) UFO and 21 (14.4%) degenerated embryos. From the total transferable embryos, 43 (51.8%)

Table 1: Simple one-way ANOVA test for Breed effects on embryo quality and developmental stages

Factor	Dependent Variable	Sum of Squares	df	Mean Square	F	Sig.
Breed of Donors	No. of transferable	1.241	1	1.241	.136	.722
	No. of unfertilized	28.269	1	28.269	50.256	.000
	No. of degenerated	.010	1	.010	.010	.923
	No. of Morula stage	.503	1	.503	.093	.768
	No. of Blastocyst stage	.003	1	.003	.001	.972
	No. of Quality 1 embryos	.741	1	.741	.162	.698
	No. of Quality 2 embryos	3.900	1	3.900	2.311	.167

Table 2: Tests of Body Condition Score Effects Across the two breeds

Factor	Dependent Variable	Sum of Squares	df	Mean-Square	F-value	Sig.
Body-condition score	No. of transferable	134.558	8	16.820	1.839	.204
	No. of unfertilized	73.856	8	9.232	16.412	.000
	No. of degenerated	20.417	8	2.552	2.500	.108
	No. of Morula stage	103.982	8	12.998	2.409	.118
	No. of Blastocyst stage	18.746	8	2.343	1.160	.420
	No. of Quality one	38.898	8	4.862	1.061	.468
	No. of Quality two	45.470	8	5.684	3.368	.053

Table 3: Tests of Breed and body condition interaction effects

Factors	Dependent Variable	Chi-Square	df	Asymp. Sig.	Exact Sig.	Point Probability
Breed: BCS	Number of transferable embryos	17.667[a]	9	0.039	0.042	0.010
	Number of degenerated embryos	23.083[b]	4	0.000	0.000	0.000
	Number of Morula stage	20.167[a]	9	0.017	0.019	0.004
	Number of Blastocyst stage	29.000[c]	5	0.000	0.000	0.000
	Number of Quality 1 embryos	16.250[d]	6	0.012	0.013	0.002
	Number of Quality 2 embryos	16.833[d]	6	0.010	0.011	0.002
	Number of unfertilized embryos	20.333[d]	6	0.002	0.003	0.001

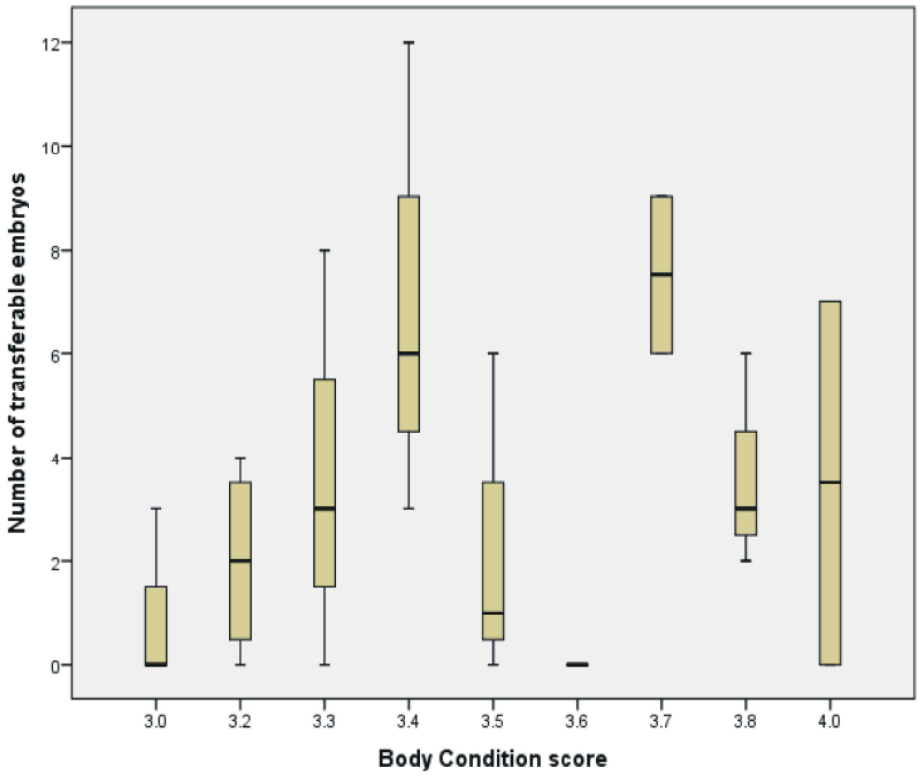

Fig. 1: Number of transferable embryos obtained from different body condition

of quality grade 1, 40 (48.2%) of quality grade 2, 58 (69.95%) morula stages and 25 (30.1%) blastocyst stage embryos. Breed effect on embryo quality and developmental stage were evaluated and only significantly affect number of UFOs (P <0.05; Table 1).

In this study, the effect of body condition on embryo quality was and has no significance. However, high number of transferable embryos was obtained from donors with BCS 3 to 3.5 of both breeds.

DISCUSSION

The quality of embryos can have a significant impact on cow pregnancy rate, which directly affects the breeding economy of dairy or beef farm. In this study, the effects donor breed on embryo quality and developmental stage was evaluated and there was no significant difference observed which agrees to previous reports by Jemal *et al.* [19]. However, there is a significant difference on number of UFOs between the two breed which agrees previous results of Degefa *et al.* [18]. This variation might indicate the presence of individual variation rather than breed influence when it comes to superovulatory treatment in bovine [20]. Some previous research reports show that, Fertilization failure in superovulated cattle is generally more pronounced, averaging approximately 45%, much lower than the present finding. It is mainly associated with poor gamete transport due to hormonal imbalances or suboptimal oocyte quality which is not uncommon in zebu breeds [21].

An average of 6 embryos and a mean of 3.5 transferable embryos were collected in the present study which is a little higher as compared to a previous result of Silva *et al.* [22] and it is much lower than the reports by Jun-Kyu Son *et al*. [23].

Environmental influences such as nutritional management, temperature and seasonality interfere with reproductive efficiency, follicular development and oocyte quality and consequently, fertility [14, 24]. Some previous studies showed that, the effects of diets and body condition score on oocyte quality and embryo development was high. In the present study, effects of body condition on embryo quality and developmental stage were on significantly different. However, high number of transferable embryos, good quality embryos was observed in donor cows with a range of 3 to 3.5 BCS. However, Kadokawa *et al.* [25] report showed that Holstein heifers with 2.75 BCS produced more excellent grade embryos than those with 3.0–3.25 BCS. Siddiqui *et al.* [16] also reports that, Zebu cattle with a mean BCS of 2.5–3.0 produced more transferable embryos (2.0 embryos) than those with a mean BCS of 4.0–4.5 (0.0) after multiple superovulation which is a little comparable with the present study. Sung-Sik Kang *et al.* [26] also reported that, Donor cows with a mean BCS of 3.7 produced more transferrable embryos than those with a BCS of 2.6 or 3.2. Finally, this study observed that donor cows with a range BCS 3 to 3.5 was ideal for superovulation.

CONCLUSSION AND RECOMMENDATION

This study concludes that, there is no such breed effects on *in vivo* produced embryo quality as well as number of transferable embryos rather under BSC or over BCS (thin/fat) of donor cows affects the quality of embryos, number of transferable embryos as well as overall embryo recovery rate. Therefore, if proper nutritional status can be maintained for donor cows on superovulation treatment by nutritional management, it is possible to enhance the utilization value of donor cows by increasing embryo recovery rate and the number of transferable embryos *in vivo* embryo production. Further, efficient production of superior embryos of high-producing donor cows could be widely applied for the improvement of high-producing dairy cows, through multiplication of cows with superior genetic by embryo transfer technology.

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