

Article

Effect of embryonic phase on pregnancy rate in embryo recipient cows

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ABSTRACT

The present report aims to analyze pregnancy rate according to the stage of embryo development at the moment of embryo transfer. All embryos had seven days of development *in vitro* and came from Nelore breed (*Bos taurus indicus*) oocyte donors. The study was performed in Uberaba, Minas Gerais, from August to October 2016. The recipient females were under the same sanitary, nutritional and hormonal protocol. In total, 443 animals of different breeds and ages have been selected. These females were used as receptors in fixed-time embryo transfer programs. Pregnancy rates of 31.95%, 42.27%, 48.16% and 56.66% were obtained for early blastocyst, blastocyst, expanded blastocyst and hatched blastocyst, respectively. The mean pregnancy rate was 44.46%. According to the obtained results, it has been possible to observe that expanded and hatched blastocysts result in better pregnancy rates in fixed-time embryo transfer.

Keywords: embryo transfer, blastocyst, gestation, *Bos taurus indicus*.

Introduction

Nelore breed has a national importance because its higher capacity of adaptating to Brazilian climate and to extensive cattle production (FREITAS, 2013). Considering that this breed has good reproductive performance, adaptation, rusticity, fast weight gain and higher genetic value, many bovine breeders and veterinarians maintain their interest (PRAXEDES et al., 2010). Consequently, Nelore cows with a higher genetic potential become oocyte donor.

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In this context, reproductive biotechnologies appeared as important tools to improve productivity and genetic upcoming in Nelore herds. The oocytes obtained from embryo donor cows are destined to *in vitro* embryo production, and the resulted embryos were transferred to recipient's cows from different origins and with lower genetic potential (SCANAVEZ et al., 2013). According to Rumpf (2007) embryo transfer and *in vitro* embryo production are both technologies available to bovine genetic improvement programs.

Embryo transfer (ET) is a biotechnology that could enhance genetic improvement and increase number of descendent from a donor cow with higher genetic value in a short period of time (NOGUEIRA et al., 2012). In this context, females with higher genetic merit for fertility, precocity and weight gain can disseminate this potential in a large scale and in a short time employing these reproductive biotechnologies which reflects directly in the increase and quality of Brazilian cattle herd (MARSON, PREVIERO and FERRAZ, 2003).

Some authors had demonstrated the influence of many factors over the pregnancy rate of embryo recipients. Among these factors are included the age of the embryo, the quality of the embryo and the synchrony between the embryo recipient and the embryo (SCANAVEZ et al., 2013; VELOSO NETO et al., 2014). Meanwhile, other authors have not observed any difference correlated to embryo stage of development and embryo quality (LOIOLA et al., 2014).

Bearing the above issues in mind, the aim of the present study was to report pregnancy rate in bovine recipients after the transfer of embryos originated from *ovum* pick up and *in vitro* fertilization from Nelore (*Bos taurus indicus*) oocyte donors in different stages of development.

Material and methods

The present study was performed in the region of Uberaba-MG, Brazil, between August 1st 2016 and October 28th, 2016 and data were obtained by a veterinarian team focused on animal reproduction and embryo transfer technique.

Four hundred forty-three (443) cows from different breed and ages were selected as recipients. These female subjects underwent to a gynecologic evaluation observing the morphologic integrity of the reproductive system and the presence of

corpus luteum (CL) in the ovary. These female subjects have also been selected according to the sanitary management – which included deworming, vaccination against leptospirosis, IBR, BVD, rabies, botulism and black leg and brucellosis exam – and the nutritional management comprising extensive pasture and mineral supplementation and water “ad libitum”.

Estrous cycles were synchronized by: D0, inserting an intravaginal progesterone device (1g, PRIMER™, Tecnopec) that remained for 8 days and by administering 2mL of estradiol benzoate (2mg, ESTROGIN™, Biofarm); D8, administration of 2mL of prostaglandin (0.526mg, SINCROCIO™, Ouro Fino), 1.5mL of equine chorionic gonadotropin (750UI, FOLLIGON™, MSD) and 0.3mL of estradiol cypionate (0.6mg, ECP™, Zoetis-Pfizer).

Oocytes were recovered from Nelore, *Bos taurus indicus*, donors by transvaginal ultrasound guided puncturing of ovarian follicles (*ovum* pick up, OPU). Next, the recovery structures have been sent to the Biovitro Laboratory (Uberaba, MG, Brazil) and submitted to *in vitro* embryo production (PIV). The resulted embryos were submitted to fresh embryo transfer (ET) after 7 days. These embryos had four different embryos stages: Bi, initial blastocyst; Bl, blastocyst; Bx, expanded blastocyst and Be, hatched blastocyst.

Embryo transfer used transcervical (nonsurgical) technique and the synchrony between the embryo recipient and the embryo has been verified by the veterinarian. It was considered acceptable a synchrony of -24h, 0 or +24h.

Recipients have been evaluated once again one day before embryo transfer to verify the presence, localization and quality of the CL. Then, CL has been classified as: R* poor with cavity, R poor, 1 good, 2 great, 3 excellent.

The data used Qui-square test and all treatments were compared at a significance level of 5% ($P < 0.05$).

Results

The obtained results are presented in Table 1.

Table 1. Number and percentage (%) of embryos from Nelore oocyte donors with 7 days of *in vitro* development classified according to their development stage. Number of pregnancies and pregnancy rate of these embryos according to their development stage.

Embryonic Stage	Animals (%)	Pregnancy (%)
Bi	72 (16.25%)	23 (31.95%) ^b
Bl	123 (27.76%)	52 (42.27%) ^{a,b}
Bx	218 (49.29%)	105 (48.16%) ^a
Be	30 (6.70%)	17 (56.66%) ^a
Total	443 (100.00%)	197 (44.46%)

*(Bi) initial blastocyst, (Bl) blastocyst, (Bx) expanded blastocyst and (Be) hatched blastocyst.

**different letters in the same column indicate significant difference ($p < 0.05$).

Discussion

The obtained results indicate the achievement of an excellent pregnancy rate, considering that pregnancy rates for embryos produced *in vitro* and transfer to recipient cows is usually around 30% in Minas Gerais region and in the present study we had 44.46% of pregnancy, which means that from 443 recipient cows, 197 became pregnant after fixed time embryo transfer (FTET). Andrade et al. (2012) reported similar results, obtaining a pregnancy rate of 37,60% with embryo produced *in vitro* and also from Nelore oocyte donors.

According to Cochito (2013) and Morais (2013) pregnancy rates around 30% are expected by veterinarians working with bovine reproduction and embryo transfer. Nevertheless, other authors have mentioned higher pregnancy rates employing zebu, Nelore or Holstein/Gir embryos (PEIXOTO et al., 2007; COSTA E SILVA et al., 2010; SCANAVEZ et al., 2013).

Considering embryo stage of development, Bi had a pregnancy rate of 31.95%, which is accordance with the expected results, but it the worst pregnancy rate achieved in the present study. Cochito (2013) obtained pregnancy rate of 28.1% for Gir embryos classified in the same stage of development, which is in accordance with the present study.

Ortlibas et al. (2015) reported a very low pregnancy rate using Bi. These authors reported only 19.57% of pregnancy after Bi transfer, however the low number of embryos transferred in this stage could have influenced the results. Controversially, Peixoto et al. (2007) have done a logistic regression analysis of factors affecting pregnancy rates of zebu embryos and observed that in general the highest pregnancy rates were obtained when early blastocysts and morula were used. However, these authors obtained embryos from a multiple ovulation program and not by PIV.

Embryos with Bl and Bx stages of development had pregnancy rates higher than the expected 30%, achieving 42.27% and 48.16%, respectively. These stages of embryo development had higher pregnancy rates. For 123 transferred embryos in Bl stage, there have been 52 pregnancies and for 218 Bx transferred embryos resulted into 105 pregnancies. Cochito (2013) reported a pregnancy rate of 54.2% for Bx, which is higher than the rate obtained in the present study. On the other hand, Ortlibas et al. (2015), for the same embryonic stage, reported a pregnancy rate of 43.53%, which is similar as the rate observed in the present study. However, lower pregnancy rates, around 30% percent, were reported for transferred Bl and Bx embryos by Colombo et al. (2010).

The embryonic phase that highlighted in the present study, with the highest pregnancy rate, is the Be with 56.66%. Similar to our study, Cochito (2013) observed higher pregnancy rate for Bx and Be embryos, 54.2% and 42.9%, respectively. These findings support that the use of embryos in these stages lead to good pregnancy rates. In another study, it was reported pregnancy rates of 35% and 30.8% for Nelore embryos in Bx and Be stages of development, respectively (Rubin, 2006).

Before embryo transfer, the recipients were evaluated to verify the presence of corpus luteum, and females with corpus luteum classified as R* and R were discarded. This decision may have contributed to a higher pregnancy rate. Colombo et al. (2010) verified a significant effect of CL on

pregnancy rate and reported that a large CL doubles the probability of pregnancy. According to Andrade et al. (2012) e Nogueira et al. (2012) the quality of the CL and the synchrony between the embryo recipient and the embryo marked a pronounced effect on the pregnancy rate. In contrast, Cochito (2013) did not observed differences in pregnancy rates dependent on the quality of the CL.

Conclusion

With the obtained results, it has been possible to conclude that the embryonic phase had influence on pregnancy rate and that the embryos classified as Bx and Be obtained higher pregnancy rates when compared to Bi. These results show that transfer of embryos in late stage of development lead to higher pregnancies rates.

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Efeito da fase embrionária sobre a taxa de prenhez de vacas receptoras de embrião

RESUMO

O presente relato buscou analisar os índices de prenhez de acordo com o estágio de desenvolvimento embrionário. Todos os embriões tinham sete dias de desenvolvimento e eram provenientes de doadoras da raça Nelore (*Bos taurus indicus*). O local da análise foi o município de Uberaba Minas Gerais no período de agosto a outubro de 2016. As fêmeas receptoras foram submetidas ao mesmo manejo sanitário, alimentar e também ao mesmo protocolo hormonal. Ao todo foram avaliados 443 animais de raças e idades diferentes, trabalhadas como receptoras na transferência de embrião em tempo fixo. Tendo a fase embrionária blastocisto inicial uma taxa de prenhez de 31,95%, blastocisto 42,27%, blastocisto expandido 48,16%, e blastocisto eclodido 56,66%. A taxa de prenhez média foi de 44,46%. Frente aos resultados obtidos, observou-se que uso de embriões em blastocisto expandido e blastocisto eclodido nas transferências de embrião em tempo fixo resultam em melhores índices de prenhez.

Palavras-chave: transferência de embrião, blastocisto, gestação, *Bos taurus indicus*.