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Short communication

Factors affecting pregnancy rates for goat embryos recovered and transferred by transcervical route

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ABSTRACT

This study evaluated the factors affecting non-surgical embryo transfer (NSET) efficiency in goats. Donor goats (n = 10) were superovulated, mated and embryos were collected by non-surgical embryo recovery 7 d after estrous onset. A total of 28 pluriparous recipient goats were subjected to short-term progestagen-based estrous induction protocol with equine chorionic gonadotropin. They received 36 fresh embryos as single (n = 20) or in pairs (n = 8) by NSET 6–7 d after estrous onset, ipsilateral to the corpora lutea (CL). Pregnancy rate was affected ($P < 0.05$) by embryo stage (blastocyst = 63.6 % *vs* morulae = 24.0 %) and quality (Grade 1 = 57.1 %; 2 = 33.3 %; 3 = 10.0 %) and not ($P > 0.05$) by the uterine horn, number of CL (1–3), lactational status and number of embryos transferred per recipient goats. Both overall pregnancy and kidding rates were 32.1 % and 13 kids were born, representing an embryo survival rate of 36.1 %. Goat embryo transfer programs can be successfully performed by using non-surgical techniques, achieving satisfactory embryonic survival rates.

1. Introduction

The Multiple Ovulation and Embryo Transfer is an assisted reproductive biotechnique that allows the multiplication of animals of high genetic value (Ledda and Gonzalez-Bulnes, 2018). The technique is based on estrus induction or synchronization and superovulation, followed by natural breeding or artificial insemination and embryo collection of donor and transfer to pre-synchronized recipient females. Embryo recovery by laparotomy and embryo transfer (ET) by laparoscopy are still techniques of choice for goats worldwide. Although positive results on non-surgical embryo recovery (NSER) have been reported in small ruminants (Fonseca et al., 2018, 2019), data related to non-surgical embryo transfer (NSET) are scarce (Candappa and Bartlewski, 2014; Fonseca et al., 2014).

Recently, goat fresh embryos resulted in satisfactory kidding rates (55 %) after NSER and laparoscopic transfer (Fonseca et al., 2018). In addition, Figueira et al. (2019) obtained a pregnancy rate (PR) of 39.4 % after laparoscopic transfer of frozen-thawed sheep embryos obtained by NSER. Before NSET recommendation, it is necessary to investigate intrinsic factors associated, including possible stress, reproductive tract injuries and efficiency of embryo deposition, and also extrinsic factors, such as corpora lutea (CL) count and quality and the uterine horn chosen, recipient parity, embryonic features (quality and stage of development) and number of embryos transferred per recipient goats. Thus, the study aimed to assess the applicability of NSER and NSET in goats, as well as the multiple factors that may contribute to NSET success.

2. Material and methods

This research was approved by the local Animal Care Committee (UFF / 0116-2011) and conducted at a commercial farm in Piau city, MG, Brazil (21° 35′S, 43° 15′ W). Healthy pluriparous Toggenburg goats with body condition score (BCS) between 2.5 and 3.5 and body weight over 35 kg were selected to be donors (n = 10 lactating does) and recipients (n = 10 lactating and n = 18 non-lactating does). All goats were maintained in a confinement system and received corn silage and chopped Napier grass (*Pennisetum purpureum*), supplemented with sugar cane. Water and mineral salt were offered *ad libitum*.

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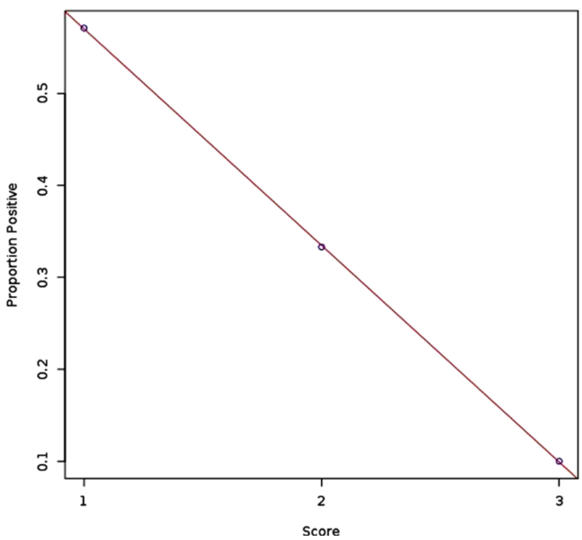

Fig. 1. Dose-response trend between the score of embryo quality and pregnancy rates. Score 1: Embryo quality 1; Score 2: Embryo quality 2, Score 3: Embryo quality 3. Slope = - 0.2358; Chi-square for slope (linear trend) = 5.6793 (P = 0.0172).

Donor goats were superovulated and NSER was performed 6–7 d after estrus onset as reported earlier (Fonseca et al., 2018). Recipient goats were submitted to a 6-d progestogen-based protocol, associated with cloprostenol and equine chorionic gonadotropin. NSET was performed as described by Fonseca et al. (2014). Donors and recipients were observed during and after NSER and NSET to detect possible signs of pain or lesions (Santos et al., 2020). Viable embryos were classified according to their stage (morulae or blastocyst) and quality (Grade 1 – excellent, Grade 2 – good or Grade 3 – fair), as reported by Stringfellow and Givens (2010).

One day before NSET, the CL of recipient goats were counted by transrectal ultrasound (Mindray DP-3300 Vet, Shenzhen, China). Twenty-eight recipient goats were equally assigned to receive fresh embryos of the same grade and stage as single (n = 20) or twins (n = 8), ipsilateral to the CL. Pregnancy was checked 23 d after NSET and kidding rate was assessed at parturition.

Variables considered as possible explanatory for PR were: number of embryos transferred per recipient (single or twins), stage (morulae or blastocyst) and quality (Grade 1–3) of the transferred embryos, number of CL (1–3) per recipient, chosen horn (right or left) and lactational status (lactating or non-lactating). The following data were recorded: PR (number of goats pregnant/number of goats receiving one or two embryos × 100); kidding rate (number of goats giving birth/ number of goats receiving one or two embryos × 100); PR according to the embryo developmental stage; embryo survival rate (number of kids born/number of embryos transferred to recipients × 100). Univariate logistic regression analyses were used to test relations between explanatory variables and the PR. Differences in proportions were assessed by Exact Fisher and Chi-square tests. The chi-square test for trend was used to assess whether there was a significant linear trend between embryonic quality level and PR. Stratified analysis was used and the Breslow-Day-Tarone test was used to evaluate if the odds ratio (OR) for strata were not homogeneous (interaction effect). All the variables with P-values < 0.10 were tested in a multiple logistic regression and then adjusted each other. The level of significance was of 0.05. The Epi Info™ version 3.5.3 software was used.

3. Results and discussion

All 28 recipient goats received compact morulae (n = 25) or blastocysts (n = 11), classified as Grade 1 (n = 14), 2 (n = 12) and 3 (n = 10). Both pregnancy and kidding rates were 32.1 % (9/28) and embryo survival rate was 36.1 % (13/36). These results are within the acceptable values, considering that PR after ET ranges from 29 % to 75 % in small ruminants (Reviewed by Ledda and Gonzalez-Bulnes, 2018). Indeed, PR of 43 % (Agrawal and Bhattacharya, 1982) and 50 % (Fonseca et al., 2014) after NSET in goats have been reported. Interestingly, Flores-Foxworth et al. (1992) compared transcervical and laparoscopic transfer in goats and observed no difference in PR (36–39 %). Although the rates are acceptable, we expect to achieve greater fertility rates after NSET as transcervical techniques are improved.

Among all the possible explanatory variables, only embryo developmental stage and quality affected PR (P < 0.05). In the univariate analysis, blastocysts had 5.7 times the efficiency of morulae (P = 0.02) and embryos of Grade 1 were 12.0 times more efficient than those of Grade 3 (P = 0.05). A significant linear trend of decreasing PR as embryonic quality scores worsened was observed, and the slope was of - 0.2358 (P = 0.02), showing a dose-response relationship between both variables (Fig. 1). In the multivariate analysis, this linear trend was also evidenced considering both embryo quality and stage (P = 0.053). There was no interaction between embryo stage and each level of quality (Table 1). These data corroborate with earlier findings when higher quality embryos resulted in significantly greater PR in sheep after both laparotomic or laparoscopic ET (Zohara et al., 2017) and greater embryo survival rate after laparoscopic ET in goats (Fonseca et al., 2018). Similarly, greater embryo survival rate was observed after laparoscopic ET of blastocyst than morulae (50 *vs* 29 %) in sheep (Armstrong and Evans, 1983) and advanced than early (90 *vs* 37 %) fresh embryos in goats. Hasler (2001) and Bari (2003) also described a significant effect of both embryo stage and quality on fertility in cattle and sheep, respectively. Overall, these data demonstrate that ET of higher quality and more advanced stage embryos may improve the uterine implantation process. In addition, it must be remembered that 69 % (25/36) of embryos transferred in the present study were morulae and only 25 % (9/36) of embryos were Grade 1. Thus, transferring embryos in more advanced developmental stage and with higher quality possibly would improve NSET success in goats.

Table 1
Univariate and multivariate analyses for the pregnancy rates of recipient goats subjected to non-surgical embryo transfer.

	Univariate model				Multivariate model	
Variable	Total	Positive (%)	OR (CI 95 %)*	P-value	OR (CI 95 %)	P-value
Embryo stage						
Blastocyst	11	7 (63.6)	5.5 (1.2–25.6)	0.02	3.5 (0.6–19.3)	0.15
Morulae	25	6 (24.0)	1.0		1.0	
Embryo quality				0.05		0.27
1	14	8 (57.1)	11.97 (1.2–121.7)	0.03	7.24 (0.6–82.5)	0.11
2	12	4 (33.3)	4.48 (0.4–48.9)	0.21	4.30 (0.4–48.7)	0.23
3	10	1 (10.0)	1.00		1.00	

Multivariate model: Score test (p-value = 0.053); Hosmer and Lemeshow test (p-value = 0.34); Breslow-Day-Tarone test for homogeneity of OR (4.72; p-value = 0.094).

* OR: Odds ratio; CI: Confidence interval.

Finally, goats presented no signs of pain or stress and no lesions or bleeding were observed during or after the procedures. Besides the benefits involving animal welfare, many advantages of transcervical techniques of embryo collection and transfer have been described (Ledda and Gonzalez-Bulnes, 2018; Santos et al., 2020). Repeated surgical procedures involving exploration of abdominal organs may cause post-operative adhesions, resulting in lower fertility (Fonseca et al., 2019). Laparotomy (33 %) was more associated with abortions than laparoscopic (0 %) ET in sheep (Zohara et al., 2017), implying that invasive techniques may lead to more deleterious effects on embryos. The current study demonstrated that non-surgical procedures for embryo recovery and transfer in goats may be an alternative to surgical traditional methods, and are aligned to the prospects of assisted reproductive techniques applied in small ruminants (Martin and Kadokawa, 2006).

4. Conclusion

Goat embryo transfer programs can be successfully performed by using non-surgical techniques, achieving satisfactory embryonic survival rates.

Declarations of Competing Interest

The authors report no declarations of interest.

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