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Pregnancy rates of frozen embryos recovered during winter and summer in Sistani cows

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Summary

During spring, summer and winter seasons, Sistani donor cows, with normal reproductive status, were superovulated and embryos were recovered non-surgically on day 7. Grade A blastocyst embryos were either transferred fresh (spring) or frozen (summer and winter). Recovered embryos during summer and winter were exposed to glycerol and frozen using conventional method. During spring season, recipient females (n = 70) were synchronized using two consecutive injections of prostaglandin $F_{2\alpha}$ analogue, 14 days apart. On day 7 after the ensuing cycle, the females were assigned into three groups to receive single embryo, either fresh (n = 14; control; recovered embryos in spring) or frozen blastocyst recovered and frozen in summer (n = 27) or winter (n = 29). Pregnancies were diagnosed by ultrasound examination, 30 days after non-surgical embryo transfer. Pregnancy rates following transfer of fresh embryos (64.3%) were higher than those that received frozen-thawed embryos (17.86%; P<0.05). There was not any significant difference between pregnancy rates of recipients receiving embryos frozen in summer (18.5%) or winter (17.2%; P>0.05).

Key words: Embryo transfer, Frozen embryos, Season, Sistani cow

Introduction

Heat stress reduced the fertility of both dairy (Wolfenson *et al*., 1988; Sartori *et al*., 2002) and beef (Dunlap and Vincent, 1971; Bigger *et al*., 1987) cattle. Detrimental effects of heat stress on embryo were established at the earlier stage of development, day 1 after AI (Ealy *et al*., 1993; Edwards and Hansen, 1997). Increase in free radical concentrations (Ealy *et al*., 1993) and a decrease in heat shock proteins (Edwards and Hansen, 1997) were considered as the main factors influencing the survival of embryo at this early stage of development. As the embryos proceed to 8 cell-stage, morula and blastocyst stage, the resistance of embryo to heat stress increased (Ealy *et al*., 1993). It is reported that *Bos indicus* is more resistance to heat stress compared to *Bos taurus* cattle (Paula-Lopes *et al*., 2003). Several studies were conducted to characterize the follicular dynamics (Niasari-Naslaji *et al*., 1999a and b), estrus synchronization protocols (Niasari-Naslaji *et al*., 2001 and 2002; Jalinous *et al*., 2006) and superovulatory response (Barati *et al*., 2006) in Sistani cattle. The objective of this study was to investigate the pregnancy rates of Sistani cows following transfer of frozen-thawed embryos recovered during summer and winter.

Materials and Methods

The investigation was conducted at Sistani Cattle Research Station, Zahak, Zabol, Sistan and Balouchestan province, Iran (Latitude: 30′ 56″ N; Longitude: 61′ 41″ E; Altitude: 483 m). Native Sistani cattle (*Bos indicus*), with normal reproductive status received a balanced ration according to NRC recommendations for beef cattle (NRC, 1990). Superovulation, evaluation and freezing of embryos, non-surgical recovery and transfer of embryos were carried out according to the procedures described previously (Curtis, 1991; Barati *et al.*, 2006). Following embryo recovery in summer and winter, Grade A blastocyst embryos were exposed to glycerol (5 min at 5% followed by 10-15 min at 10% glycerol) and frozen using a controlled embryo freezer (Freeze Control, CL-550, Cryologic, Australia). The embryos were cooled at the rate of 2°C min^{-1} to -6.5°C, at which the embryos were maintained for 5 min and then seeded. After an extra 10 min at this temperature, the embryos were cooled at the rate of 0.5°C min^{-1} to -35°C, when the embryos were plunged into liquid nitrogen. The embryos were thawed for 15 sec in air followed by 15 sec in a 35°C water-bath. Step-wise dilution of embryos was performed at 6, 3 and 0% glycerol in association with 10% sucrose. The embryos were recovered and frozen in summer (July; temperature: 38.3°C; relative humidity: 15.73%) and winter (February; temperature: 20.04°C; relative humidity: 35.3%) were transferred in spring (April; temperature: 28.9°C; relative humidity: 24%). Fresh embryos recovered during spring and transferred non-surgically, served as the control. Sistani cattle (n = 70; 342.0 ± 14.5 kg live weight; 42.0 ± 8.5 months of age) were used as recipients. The estrous cycle was synchronized using two prostaglandin $F_{2\alpha}$ analogue injections (Prosolvin, Intervet, the Netherlands) 14 days apart. Estrous behaviour was monitored from 24 to 96 hrs after the second prostaglandin $F_{2\alpha}$ injection. On day 5 of the estrous cycle, the presence and location of *corpus luteum* was determined using an ultrasound scanner equipped with 5 MHz linear probe (Aloka 500, Japan). On day 7 after standing estrus, females were divided into 3 groups and received fresh embryos (Control; n = 14), or the embryos frozen in summer (Group 1; n = 27) or winter (Group 2; n = 29). Pregnancy was diagnosed on day 30 using ultrasonography, and pregnancy rates were analysed using the chi-squared test in SAS (SAS, 2001).

Results

The pregnancy rates following the transfer of fresh embryos (9/14; 64.3%) were significantly higher than that of frozen-thawed embryos (10/56; 17.86%; P<0.05). There was no significant difference in the pregnancy rates of the recepients receiving embryos frozen in either summer (5/27; 18.5%) or winter (5/29; 17.2%; P>0.05; Table 1).

Table 1: The pregnancy rates following transfer of fresh embryos (recovered in spring) or the embryos that were frozen in summer and winter in Sistani cattle. All transfers carried out in spring

Embryo	Number of transfer	Number of pregnant	Pregnancy rates (%)
Fresh	14	9	64.3
Frozen (Summer)	27	5	18.5
Frozen (Winter)	29	5	17.2

Discussion

The present study examined the difference in pregnancy rates of Sistani cattle after transferring fresh embryos and the embryos frozen in summer and winter seasons. There was significant reduction in the pregnancy rates of frozen-thawed (17.86%) compared to those of fresh embryos (64.3%; P<0.05). Hasler (2001) reported the pregnancy rates of 68.3% out of 9023 fresh embryo transfer, which is consistent with the result of transferring fresh embryos in the present study. The pregnancy rates following transferring frozen-thawed embryos were 56.4% (n = 3616) in Holland (Hasler, 2001), 58.4% (n = 5297) and 68.7% (n = 774) in two separate studies in USA (Hasler, 2001). Martínez *et al.* (2002) reported the pregnancy rates of 40.4% following transfer of frozen-thawed embryos in cows. They demonstrated that there was a relationship between equilibration time (exposure time to cryoprotectant) and the stage of embryonic development. In their study, the pregnancy rates of frozen-thawed blastocyst (20%) were lower than morula (59%) when the equilibration time was fixed at 10 min (Martínez *et al.*, 2002). They have shown that reducing the equilibration time to 5 min enhanced the pregnancy rates following transfer of frozen-thawed blastocyst. The more developed embryo has smaller size blastomers with increased permeability to cryoprotectants. This in turn, reduces the length of time required for exposing embryos to cryoprotectants (Széll *et al.*, 1989; Rayos *et al.*, 1992a and b). Therefore, part of the reduction in pregnancy rates in this study may be due to the stage of embryonic development (blastocyst). In the present study, there was no significant difference between pregnancy rates of transferred embryos frozen in summer (18.5%) and winter (17.2%). There is no study demonstrating the effect of season on the pregnancy rates following transfer of frozen-thawed embryos (Hasler, 2001). However, one study demonstrated that season did not have any effect on fertility of dairy cattle following transfer of fresh embryos (Putney *et al.*, 1989). More recently, we have demonstrated that Sistani cattle are able to maintain their body temperature below the critical point (39.6°C) during hot summer (Barati *et al.*, 2006). *Bos indicus* breeds, including Sistani cattle, have a greater thermo-tolerability due to a better thermal regulatory response to high ambient temperature. This is due partially to a lower internal heat production (Seif *et al.*, 1979) as a consequence of a lower basal metabolic rate (Johnston *et al.*, 1958), the ability to have a greater heat loss due to larger sweat glands (Pan, 1963), and diversion of blood flow from the body core to the skin (Finch, 1986). The capability of *Bos indicus* cattle to adapt to environmental elevated temperature may explain in part their better estrous expression and a higher conception rate during the summer months (Wilson, 1946; Randel, 1984; Lamote-Zavaleta *et al.*, 1991). To the best of our knowledge this is the first report comparing the pregnancy rates following transfer of frozen-thawed and fresh embryos in *Bos indicus*, and Sistani cattle in particular. In conclusion, the pregnancy rates of Sistani cattle following transfer of frozen-thawed embryos was not compromised by heat stress. More research is required to elaborate the best possible method of embryo cryopreservation in Sistani cattle.

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