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Factors affecting the pregnancy rates post embryo transfer in dromedary camels: Quality and number of recovered embryos

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ABSTRACT

The present study was carried out to evaluate the effect of embryo quality, and number of embryos recovered per flush from donors on the pregnancy rates in dromedary camels. Donors (n=51) were flushed at day 8-9 after breeding with 1000 ml commercial flushing media prior transfer to synchronized recipients (n= 457) using transvaginal technique. Pregnancy was diagnosed at Day 18-19, 30 and 60 post-transfer to recipients at rate of 55.1%, 47.9%and 43.4%, respectively. Transferring transparent embryos resulted in significantly high pregnancy rates at Day 18-19 and 60 (P<0.001) and Day 30 (P<0.0001) compared with those of semitransparent and dark embryos. Transferring of spherical embryos resulted in significantly high pregnancy rates at Day 30 (58.6%; P<0.003) and Day 60 (52.7%; P< 0.001) compared to folded embryos. Neither the size of embryo nor number of recovered embryos had significant effect on pregnancy rates after transfer. In conclusion, higher pregnancy rates up to Day 60 after transfer could be obtained if transparent and spherical embryos are implemented in embryo transfer protocol in dromedary camels.

1. INTRODUCTION

Camelids are seasonal breeders (Adams et al., 1990) and induced ovulators (Novoa, 1970). Unfortunately, the reproductive performance of camels under natural condition is extremely low when compared with other farm animal species (Aboul-Ela, 1991; Skidmore, 2003). This low reproductive performance is considered as the most crucial factor affecting camel productivity (Tibary et al., 2005; Hemeida, 2014). The interest of enhancing the camel reproductive efficiency has increased in last few decades due to the development of camel racing industry in the Middle East (Skidmore and Adams, 2000; Nowshari et al., 2005). Camelids are probably the last large domestic species to experience the benefit from assisted reproductive technologies (ART) (Anouassi and Tibary, 2013; Nagy et al., 2013; Skidmore et al., 2013). Success of embryo transfer (ET) is achieved by adjusting factors to increase pregnancy and decrease pregnancy loss rates (Vettical et al., 2016; Karen and Mansour, 2020).

According to Karen and Mansour (2020) the quality of embryo, namely shape and size, had significant effects on pregnancy and pregnancy loss rates. Transferring spherical embryos significantly increased the pregnancy rates at days 19 and 60 (58.5%) and (37.6 %) compared with folded shaped ones (49.3 and 29.7%, respectively). By increasing the embryo size, the probability of the embryonic mortalities increased significantly.

The number of recovered embryos impacts the pregnancy rate outcome where increasing recovered embryos (more than 4 embryos) upsurges the probability of pregnancy rate (McKinnon et al., 1994; Karen and Mansour, 2020). The current study was conducted to evaluate the effect of embryo quality (transparency, shape, and size) and number of recovered embryos on the outcome of the pregnancy rates.

2. MATERIAL AND METHODS

2.1. Animals

The current work was carried out at Tharb Camel Hospital, State of Qatar, in two successive breeding seasons (October to April, 2017-2018), (October to February, 2018-2019). The valuable donors (n=51) and recipients (n=457) used in the present study were from 5 to 14 years which gave birth previously and were negative for Brucella, and trypanosomiasis and other problems affect reproductive soundness(Seidel and Seidel, 1991; Tibary and Anouassi, 1997; Tibary et al., 2001; Anouassi and Tibary, 2013). Rhodes grass, wheat bran and dried alfalfa were the main components of camel ration, and water and mineral blocks were supplied with free access. They were kept in separate pens with fence, each pen had about 40 to 50. Thirteen camel bulls belong to the center were used for mating of the donors.

2.2. Superovulation and breeding of the donors

Ultrasonography was carried out using trans-rectal technique in all she-camels by using Ibex Evo® scanner (E.I. Medical Imaging, Colorado, USA) supplied with a 6 to 8MHz linear rectal probe. Superovulation of the donor was applied according to Skidmore et al. (2002).

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Briefly, the donors having a follicle(s) with a diameter between 12 and 16 mm were administered (20 µg) GnRH (Buserelina®, Zoovet, Argentina) to induce ovulation. After 3 to 4 days, ovulation was confirmed by trans-rectal ultrasonography, then the superovulation protocol was started using a combination of single administration of 2500 IU eCG (Folligon®, MSD Animal Health, Canada) and 400-440 mg porcine FSH (Folltropin V®, Bioniche Animal health, Canada) every 12 hours in decreasing doses (2 × 80 mg, 2 × 60 mg, 2 × 40 mg, 2 × 20 mg) for 4 days which might be extended to 6 days by injecting (2 × 20 mg of FSH) two days when the response at day 4 was poor (only few follicles grow (5-8 mm). The donors received 500 µg Cloprostenol (Estrumate®, MSD Animal Health) at the last dose of FSH. Thereafter, follicles size was examined using an ultrasound and when the majority of follicles reached 12 to 16 mm in diameter, the donors were introduced to one of the bulls two times at 12-hrs interval for mating then received 3000 IU hCG (Chorulon®, MSD Animal Health) just after mating for maximum induction of ovulation due to presence of (8-25 follicle) on the ovaries and mating only is not enough for induction ovulation to all follicles. Mating day was designated as Day 0 for calculating the embryo collection day and fetus age after the embryo was transferred to the recipient.

2.3. Embryo collection and evaluation

Non-surgical embryo collection was performed in a standing position after 8 or 9 days of donor mating according to Tibary and Anouassi (1997). Briefly, after the vulva was dry cleaned and disinfected using alcohol 70%, an 18-22-gauge Foley catheter (Bioniche, Canada) was placed at the base of the uterine horn using recto-vaginal technique. The cuff of the Foley catheter was inflated with 30-45 ml air and flushing of each horn was performed repeatedly with 30-70 mL commercial flushing medium according to size of the horn (Euroflush®, IMV Technologies, France). Total amount of flushing medium for each horn was about 1000 ml. After recovering of the flushing medium into a sterile embryo filter through Y tube (IMV Technologies), the filter was taken inside the lab then the remaining filtrate inside the filter was transferred in a gridded plate for embryo (s) searching under a stereomicroscope with a 3.2 mega pixel digital camera (Wesco WS7, California, USA). The hatched blastocysts were washed for 4 to 5 times in a 5 well plate containing commercial holding medium (REF. 019449, IMV Technologies). The number of embryos was counted, and the diameter was measured by taking two diameters then calculating their average by Wescometrics software (Wesco). Embryos were divided into three groups regarding their diameter into small embryos (300- 450 µm in diameter), medium (451-750 µm in diameter embryos) and large embryos (751-1200µm in diameter) and into three different shape categories [spherical, folded embryos (which characterized by signs of partial collapse and shrinkage in their outer cell mass) and spindle embryos which were lost the majority of their blastocele with signs of total collapse and shrinkage taking leaf like shape)] (Abd-Elfattah et al., 2020). The number of recovered embryos was classified into two groups: 1 to 4 and > 4 embryos, same classification was described by Karen and Mansour (2020).

2.4. Treatment of recipients and embryo transfer

Induction of ovulation was performed by injecting 20 µg Buserelin acetate to those recipients having a follicle 12 to 16 mm in diameter after 24- 48 hrs of donors mating. Ovulation was confirmed after (6-7 days after induction of ovulation) i.e., one day before embryo transfer either using serum progesterone test and /or by CL was detected using ultrasonography.

Embryos in holding medium were individually loaded into a 0.25 ml straw. Then using special embryo transfer gun, the embryo was deposited into the anterior tip of the left uterine horn using recto-vaginal technique (Abd-Elfattah et al., 2020).

2.5. Blood sampling and progesterone

Collection of blood sample was done one-day before embryo transfer (ET) from recipients' jugular vein for confirmation of ovulation then again after 10 days after ET for diagnosis of pregnancy as the CL starts its regression after (9 to 12 days) after induction of ovulation if there is no embryo to induce the maternal signals of pregnancy which maintain the CL. The collected blood samples were centrifuged at 1500 ×*g* for 15 minutes for sera separation. Concentration of progesterone in the fresh sera of the recipients and donors were measured with a commercial assay kit (Elecsys Progesterone III for Cobas e 601, Roche Diagnostics GmbH, D-68305 Mannheim) using electro chemiluminescence immunoassay (ECLIA) according to Ayad et al. (2014). The minimum detection limit of the assay was 0.05 ng /mL. The inter- and intra- assay coefficient of variations for low (2 ng/mL) and high (7.84 ng/mL) P4 concentrations were 3.72%, 4.77% and 8.1%, 0.9 %, respectively. In the previous observations in our biochemistry lab at Tharb Camel hospital for pregnant she camels the highest specificity and sensitivity of P4 test were detected when P4 level was ≥2 ng/mL so that, we used it as a cut-off value.

2.6. Diagnosis of pregnancy

The first detection of pregnancy was done 10 days after ET (i.e., about 17- 18 days after induction of ovulation) by collection of blood sample for estimating blood P4 level. Pregnant recipient has progesterone level ≥2 ng/ml and they showed a very important sign by their tail called tail cocking (erected and coiled tail) when approached by a camel bull (Tibary and Anouassi, 1997). Re-examination to the pregnant females was performed using ultrasound at one and two months of pregnancy to detect any pregnancy losses. Recipient was considered as non-pregnant at day 30 and 60 if the fetus with the surrounding fetal fluids was not detected (absence of pregnancy) the fetus was detected without its heartbeat (early embryonic death).

2.7. Data calculation and statistical analyses:

Pregnancy rates were calculated by dividing the number of conceived recipients at day 18-19 and positive pregnant recipients at days 30 and 60 after donors mating by the total number transferred embryos conducted x 100. Pregnancy loss rates were calculated by counting the number of recipients lost their pregnancy after they were diagnosed positive at day 18-19 then divide the number of lost pregnancies by the total pregnant at day 18-19 conducted x 100. Binary logistic regression analysis was used to evaluate the relationship between pregnancy (dependent variables) and number of recovered embryos per donor flush, and shape, transparency, and size of the embryos (independent variables). Probability of pregnancy for any risk factor was calculated by dividing 1 by the odds ratio of this risk factor **(1/ odds ratio**). If the odds ratio of the

independent variable was higher than 1, this indicates an increase the probability of occurrence of pregnancy at Days 19 or 60 or the pregnancy losses and vice versa if the ratio is less than one. The Chi-square procedures with Yate's continuity correction was used to compare proportions whenever required. The binary logistic regression was applied using IBM SPSS Statistics 20®.

3. RESULTS

Out of 829 embryo transfers into recipients, 457 (55.1 %), 397 (47.9 %) and 360 (43.4%) pregnancies were diagnosed at Day 19, 30 and 60, respectively. Out of 457 diagnosed pregnancies at Day 19, 97 pregnancies were lost between 19 and 60 days of pregnancy with a pregnancy failure rate of 21.23% (97/457, Table 1).

Table 1 Pregnancy rates at Day 19, 30 and 60 after embryo transfer in dromedary camels

Day of pregnancy diagnosis (Method)	Number of pregnant recipients	Pregnancy rate (%)	Pregnancy failure rate (%)
Day 19 (P4 level)	457	55.1	--
Day 30 (US)	397	47.9	13.1
Day 60 (US)	360	43.4	21.2

P4= progesterone *US*= Ultrasound

Regarding the effect of transparency, size, shape, and number of recovered embryos on the pregnancy rate at 10 days post ET (Day 18 to 19 after donor mating), transparency of embryo was the only parameter had a significant effect (P< 0.05) on pregnancy rate. Based on binary logistic regression analysis, transferring semitransparent and dark embryos into recipients reduced the probability of occurrence of pregnancy by 1.58 (1/0.632) and 2.14 (1/0.468) times, respectively, when compared with that of transparent embryos (Table 2).Both spherical shaped embryos in shape category and transparent embryos in transparency category had positive significant effects on the pregnancy rates at days 30 (P< 0.01 and P< 0.0001 respectively; Table 2) and 60 (P< 0.005, Table 2) of pregnancy. Transferring folded-shaped embryos into recipients reduced the likelihood of occurrence of pregnancy at day 30 by 1.6 times (1/630; Table 2) and at day 60 by 8.7 times (1/0.115 Table, 2) when compared with spherical-shaped embryos. Also, transferring semitransparent and dark embryos into recipients reduced the occurrence of pregnancy at day 30 by 1.6 (1/0.639) and 3 (1/0.339) times and at day 60 by 1.6 (1/0.617) and 2.2 (1/0.446) times, respectively, when compared with transparent embryos (Table 2). On the other hand, neither the size nor the number of recovered embryos had any effect on the pregnancy rates at days 18 - 19, 30 and 60 of pregnancy (Table 2). No interaction was observed among the independent variables (Transparency x Shape, Transparency x Size, and Transparency x No. of recovered embryos) or (Shape x Size and Shape x No. of recovered embryos) or (Size x No. of recovered embryos) on pregnancy rates.

Table 2 Effect of quality and number of recovered embryos on the pregnancy rate at Day 18-19, 30 and 60 post-mating of donor dromedary camels (*n*=829)

Risk factors	Category	Day 18-19		Day 30		Day 60	
		Pregnancy rate (%)	Odds ratio*	Pregnancy rate (%)	Odds ratio*	Pregnancy rate (%)	Odds ratio*
Transparency	Transparent	225/348 (64.7)*	Referent	204/348 (58.6)*	Referent	188/348 (54.0)*	Referent
	Semitransparent	173/340 (50.9)*	0.632	149/340 (43.8)*	0.639	130/340 (38.2)*	0.617
	Dark	59/141 (41.8)*	0.468	44/141 (31.2)*	0.399	42/141 (29.8)*	0.446
Shape	Spherical	223/364 (61.3)**	Referent	206/364 (56.7)*	Referent	192/364 (52.7)*	Referent
	Folded	208/406 (51.2)**	0.759	171/406 (42.1)*	0.630	149/406 (36.7)*	0.115
	Spindle or elongated	26/59 (44.0)**	0.696	20/59 (33.9)**	0.579	19/59 (32.2)**	0.617
Size	Large	144/253 (56.9)**	Referent	125/253 (49.4)**	Referent	110/253 (43.5)**	Referent
	Medium	204/354 (57.6)**	0.978	181/354 (51.1)**	0.984	166/354 (46.9)**	0.978
	Small	109/222 (49.1)**	0.850	91/222 (4.1)**	0.844	84/222 (37.8)**	0.850
Number of recovered embryos	< 4 embryos	153/296 (51.7)**	Referent	138/296 (46.6)**	Referent	128/296 (43.2)**	Referent
	> 4 embryos	304/533 (57.0)**	1.179	259/533 (48.6)**	1.014	232/533 (43.5)**	0.938

*If *P value* < 0.05, this indicates there is a significant difference. **If *P value* >0.05, this indicates there is a non-significant difference
If the odds ratio of risk factor > 1 and *P* < 0.05, this indicates an increase in the probability of occurrence of pregnancy. If the odds ratio of risk factor < 1 and *P* < 0.05, this indicates a decrease in the probability of occurrence of pregnancy

4. DISCUSSION

The present field study demonstrated that using some simple obvious criteria such as embryo shape and transparency, embryo transfer outcome could be predicted reliably. Transferring of transparent embryos resulted in significant higher pregnancy rates at days 18 -19 to 60 when compared with dark and semitransparent ones. A higher pregnancy rate (61.3%) was obtained when only spherical embryos were transferred. Our results are similar to that recorded by Skidmore et al. (2002)(10/15, 67%). Pregnancy rates recorded at days 18-19 (55.1%), 30 (47.9%) and 60 (43.4%) after transfer of dromedary camel embryos, were within the range obtained at the same days of pregnancy in previous studies (Anouassi and Tibary, 2013; Karen and Mansour, 2020).

In addition, the pregnancy failure rate between days 19 and 60 (21.23%) was similar to that reported by Tinson et al. (2012) and Abd-Elfattah et al. (2020). While it was much lower than that reported by Karen and Mansour (2020) which may be attributed to the difference in the age of donors and recipients, the year of the season and quality of transferred embryos (Tinson et al., 2012; Anouassi and Tibary, 2013; Karen and Mansour, 2020). Moreover, in the current work the lower rates of losses might be explained by the early pregnancy diagnosis using P4 estimation at Day 18-19 pregnancy. As some times, the CL was detected using ultrasound and fluid, but the progesterone level was lower than 2 ng and in those cases, it need urgent injection of progesterone (50- 100 ng) to maintain the pregnancy.

Number of pregnant recipients at day 30 to 60 were significantly increased when spherical embryos were transferred to them. Increase sensitivity of folded embryos to handling which might be due to is due to the stress of manipulation boosted oxidative stress which greatly affected the bad quality embryo (other than spherical shape embryo)

At Day 30, those recipients which was pregnant after transfer of spindle and elongated embryos lost 30% of the pregnancy which was significantly higher than folded embryos (18%). At Day 60, the pregnancy loss rate of spindle or elongated embryos markedly decreased (5%). The cause of higher early embryonic loss between spindle and elongated embryos then followed by decrease, may be due to maternal recognition of pregnancy which leads to maintenance of CL for longer period (10 days after transfer) then failed to develop and implant into endometrium. Those embryos who succeeded to implant were sound enough to complete the full term of pregnancy.

There was no effect of the size of the embryo on the pregnancy rates after transfer. Similar result was reported

in dromedary camel, whereas the pregnancy rates were similar after transfer of cooled camel embryos with small and large diameters (Abd-Elfattah et al., 2020). Although transferring of medium and large sized embryos markedly increased pregnancy loss rates after ET compared to small sized ones, the effect of size on pregnancy rates showed a non-significant effect (Karen and Mansour, 2020).

Number of recovered embryos per uterine flush showed non-significant effect on pregnancy rate according to the present study at days 18 to 60. This result was inconsistent with two previous studies in dromedary camel (McKinnon et al., 1994 ; Karen and Mansour, 2020). Transferring of embryos resulted from donors from which more than four embryos recovered led to increasing pregnancy rates at day 19 (Karen and Mansour, 2020) and day 19 to 23 (McKinnon et al., 1994). The non-significant results at day 19 might be due to the small number of embryos in the groups compared with the (McKinnon et al., 1994; Karen and Mansour, 2020).

5. CONCLUSION

From the present study it could be concluded that higher pregnancy rates could be obtained if transparent embryos are transferred. Moreover, the pregnancy rate after ET might be affected by the shape of embryos.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest for current data

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