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Hormonal management of ovarian activity in breeding camels two months ahead of the natural breeding season

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Abstract

Early ovarian activity in camels is rewarding for camel breeders on account of better growth in calves born to early bred females. The objective of this study was to stimulate ovarian activity in breeding camels two months ahead (September-October) of the natural breeding season (November-April) for early conception. Ovarian follicular growth and maturation was stimulated during two years (2010 and 2011) in camels (from 16 September) not evidencing any follicle growth on 4 consecutive transrectal ultrasonographic (TRUS) examinations (every 4 days between 1-15 September) by either im administration of a low dose eCG (2000 IU) (n=43) or an Ov-Synch (n=39) protocol (GnRH + PG + GnRH on 0, 7 and 9 days). Camels with a persistent CL (n=20) and ovarian cysts (n=8) were treated by im administration of 500 µg of a prostaglandin (Estrumate) and 4500 IU im of hCG (Chorulon) respectively. Thirty camels were not given any treatment and kept as control. All treated and untreated camels were sequentially examined for a maximum of 10 times every 4 days by TRUS and mated with males on visualization of a mature follicle (1.0-2.0 cm) over the ovaries. A high proportion of eCG treated (79.06%) and Ov-Synch treated (71.79%) camels evidenced a mature follicle at day 8 and 12 of treatment respectively whereas 75.05 and 75.0% of PG treated and hCG treated camels evidenced a mature follicle on day 8 and 12 of treatment respectively. Only 26.66% of untreated control camels evidenced a mature follicle towards the end of the study period (October) in majority (62.5%) of camels. The pregnancy rates were 50.0%, 65.11%, 61.53%, 37.50% and 50.0% in control, eCG treated, Ov-Synch treated, hCG treated and PG treated camels respectively. The efficiency of pregnancy diagnosis by tail cocking, TRUS and serum progesterone was high at day 20 post mating but accurate at day 30 post mating because of 9.21% early embryonic deaths that occurred between day 20 and 30 post mating. It was concluded that a small proportion of camels evidence a mature follicle between September and October months and follicle growth and conception can be stimulated two months ahead of the natural breeding season in camels by the use of either eCG or Ov-Synch treatments. Camels evidencing a persistent CL or ovarian cysts should be treated with PG or

hCG for obtaining follicle growth and pregnancy. Camels evidencing serum progesterone profiles above 2.0 and 3.0 ng/mL at 20 and 30 day of mating should be considered pregnant.

Keywords: Camel, eCG, hCG, Ov-Synch, PG, pregnancy diagnosis.

Introduction

Camels are raised in UAE for heritage and races. Although the reproductive season in camels in UAE continues from November to April (Abou-Ela, 1994 and Tibary and Anouassi, 1996) breeders often tend to breed camels early in the season for reasons of better growth in calves (born between November to January) born to early bred females. The pregnancy rates during early season tend to be poor because of transition from summer anestrus and poor follicle growth during this period (Sghiri and Driancourt , 1989). Camels maintained for race are fed judiciously (Afzal and Sakkir, 1994) to restrict body weight for enhanced performance. Race winning females are desirable candidates for becoming pregnant in the breeding season that follows but these females often evidence poor follicle growth during the breeding season (Tibary and Anouassi, 1997) and veterinarians are often confronted with initiating follicle growth. Ovarian cysts have been recorded in breeding camels with incidence of 0.9% to 14.0% (Musa, 1984; Al-Ani et al., 1992; Ali *et al*., 2010 and Hegazy *et al.*, 2004) and such animals tend to be problematic breeders (Ali *et al*., 2010 and Vyas *et al*., 1998).. Follicular growth in camels two months ahead (transition phase) of the breeding season is limited to appearance of small follicles (below 4 mm) appearing at the periphery of the ovaries sometimes referred as "black periphery" (Dholpuria *et al*., 2012) and only follicles above 4 mm are clearly visible and measurable with ultrasound (Dholpuria *et al*., 2012 and Vyas *et al*., 2008). The small sonographically visible follicles rarely attain the size of a mature follicle (1.0-2.0 cm) during the non-breeding season (Manjunatha *et al*., 2012) or the transition phase (Sghiri and Driancourt , 1989). The season affects the growth of the dominant follicle during the dominance period however; the other characteristics of the follicular wave are not affected (Manjunatha *et al*., 2012). During the breeding season clearly visible mature follicles appear over the ovaries (Vyas *et al*., 2008). The follicular diameter that is considered mature and optimum for mating in camels is 1.0-2.0 cm (Skidmore *et al*., 1996). Hormonal therapies for reproductive management in camels have been mentioned, yet the approaches for early induction of cyclicity in camels are limited and not used widely. Approaches to augment early follicle growth include use of eCG (Agarwal *et al*., 1996 and 1997), GnRH (Bono *et al*., 1991 and Ismail *et al*., 1998), the use of face mask (Vyas *et al*., 2008). or melatonin implants (Dholpuria

et al., 2012). This manuscript describes hormonal approaches for inducing ovarian activity and pregnancy in camels two months ahead of the natural breeding season.

Materials and Methods

1. Experimental animals

Non-lactating Arabian camels (*Camelus dromedarius*) that had parturated at least once and reared at the Bin-Hamoodah Agricultural premises, Al-Ain (Latitude 24° 16' N; Longitude 55° 36' E), UAE were included in the study. All camels at the farm were tested for the presence of Brucellosis and were found to be brucella free. Breeding camels were fed ad lib oat hay, lucerne and a commercially available concentrate at the rate of 1500-2000 gm/day. Water was provided ad lib to the camels. The age of camels used for breeding ranged from 6 to12 years. Camels were routinely given an anthelmintic, albendazole (Vermizole Vet and Agric Products Mfg Jordan) administered at the rate of 80-120 mL per camel. Camels were maintained in an open paddock with fenced area and were given regular exercise within the area. During the hot day hours camels were kept in shades.

2. Hormonal treatments

Camels not showing any follicular activity on 4 consecutive transrectal ultrasonographic (TRUS) examinations and not showing any receptivity towards males (1-15 September 2010 and 2011) were randomly treated (Starting on 16 September) with either an im injection of 2000 IU of eCG (Folligon, Intervet, Holland) (n=43) or an Ov-Synch protocol (n=39). Thirty camels were kept as untreated control. In the Ov-Synch protocol camels were treated with an im administration of 5 mL of GnRH (Receptal, Intervet, Holland) on the day of examination (Day 0) followed by an im administration of 500 µg of a prostaglandin (Estrumate, Schering Plough Animal Health, Germany) on day 7 and 5 mL im of GnRH on day 9 as described for cattle (Stevenson *et al*., 1999). Camels with ovarian cysts (n=8) were treated with 4500 IU im of hCG (Chorulon, Intervet, Holland). Camels with a palpable and sonographically visible persistent corpus luteum were treated with 500 µg of a commercially available prostaglandin (Estrumate, Schering Plough Animal Health, Germany). All treated and control camels were brought near males for estrous behaviour and examined every 4 days by TRUS for a maximum of 10 examinations to monitor the appearance of follicle.

3. Ultrasonography

Camels were restrained in a chute during the early morning (7 AM) or evening (7 PM) hours after administration of xylazine (Rompun, 2ml IV, 20 mg/mL) in a standing position. The examiner stood on a wooden dais for examination. Vicious camels were sedated similarly but examined in a sternal recumbency by tying both fore legs and hind legs separately. Transrectal ultrasonography

was performed using a dual frequency (5-10 MHz) linear array probe (Prosound 2, Aloka, Japan) for the presence of an ovarian follicle as per previously described methods (Vyas *et al*., 2008) with some modifications. Briefly, the examiner introduced his hand protected with a disposable sleeve inside the rectum and evacuated the rectum of the feces. The probe of the ultrasound protected with a sleeve and containing the ultrasound gel (Bromed, Brosco International USA) was introduced in the rectum. The probe was moved inside and the entire genital tract including both the ovaries was examined. The data was recorded regularly. Anechogenic structures appearing on the ovaries were considered follicles. Follicles above 4-5 mm were measured by the inbuilt callipers. Follicles were considered mature when they had attained a size of 1.0 – 2.0 cm and mating was given to camels attaining this follicle diameter. Mated camels were examined at 20 and 30 days post mating for evidence of pregnancy. The camel was considered pregnant if anechogenic conceptus fluid with an echogenic embryo and the embryonic heart beat was visible sonographically as described previously (Skidmore, 2000 and Vyas *et al*., 2002). Disappearance of a sonographically visible (day 20) conceptus fluid and embryo at day 30 and a decline in serum progesterone was considered an early embryonic death. The presence of an exceptionally large sized (>2.5 cm) follicle with a spoke wheel appearance and echogenic material inside without evidence of pregnancy was considered an ovarian cyst as described previously (Tibary and Anouassi, 1997). A CL was considered persistent when it was evident on 5 sonographic examinations without evidence of pregnancy and tail cocking (Shalash and Nawito, 1964 and Tibary and Anouassi, 2000).

4. Breeding

Female camels evidencing receptivity towards male camels were mated to virile stud camels with proven fertility when a mature follicle (1.0 to 2.0 cm) was visible sonographically. Two matings were given with studs at 12 hourly intervals. Mated camels were examined again after 2 days and if the follicle continued to exist they were re-mated.

5. Progesterone assay and cocking behaviour

Camels were evaluated for cocking of tail in the presence of a male at 15-20 days of mating. Blood (5-10 mL) was collected by jugular veinepuncture from all mated camels at day 20 and 30 post mating in vacutainer (vacuette) tubes and analyzed for serum progesterone using Enhanced Chemiluminescence Immunoassay (ECLIA) System (China Medical Technologies) at a commercial laboratory. Values above 2 ng/mL were considered positive and values below 1ng/mL were considered negative at 20 days of pregnancy whereas camels with values between 1 to 2 ng/mL were considered doubtful (Skidmore, 2000)

and were confirmed pregnant by the laboratory if the values increased above 2.5 ng/mL on samples tested at day 30 of mating. As mentioned by the laboratory the inter and intra assay coefficient of variation were below 10 percent.

6. Statistical analysis

Data of both the years (2010 and 2011) was pooled to calculate the results of the treatments. The differences between treatments were compared statistically using student't' test.

Results

1. Follicle growth in untreated camels

None of the camels evidenced a mature follicle between 1 to 15 September 2010 and 2011. Out of 30 untreated (control) breeding camels only 8 camels (26.66%) evidenced a mature follicle during the study period. The mature follicle was evident in 37.5% (3/8) camels by the end of September and in 62.5% (5/8) camels by 24 October. A small proportion (23.33%) of the camels (n=7) did evidence small follicles (less than 4 mm) at the periphery of the ovaries visible as small black structures but mature follicles were not visible during such examinations. Half (50.0%) of the untreated camels did not evidence any follicle growth during the study period.

2. Effect of eCG treatment on follicle growth

Out of total 43 camels treated for inducing early follicle growth only 38 (88.37%) camels evidenced follicle growth within 4 days of treatment. The mature follicle was found on either of the ovaries in 34 (79.06%) camels at 8 days which were then mated. In 11.62% (5/43) camels no follicle was seen during the study period and in 10.52% of camels (4/38) which evidenced growing follicles the follicle did not grow further to reach a mature ovulatory size (1.0-2.0 cm). Subsequent regular examination of these camels revealed that 2 of these treated camels developed anovulatory ovarian cysts.

3. Effect of Ov-Synch protocol

Follicle growth was seen only in 4 camels treated with Ov Synch at the time of first examination whereas at the time of second examination at 8th day 79.48% (31/39) camels showed follicle growth but at day 12 a mature follicle was evident in 71.79% (28/39) camels. In 9.69% (3/39) camel's follicle growth was evident at day 8 of treatment but a mature follicle was not seen during the study period. Eight camels (20.51%) did not evidence any follicle growth during the study period.

4. Effect of PG treatment

Camels with a corpus luteum (CL) and treated with a PG evidenced the appearance of a mature follicle within 4 days. Out of the total treated camels 75.0% (15/20) camels evidenced a mature follicle at day 8, and 5 camels showed small follicles (4-5 mm) which did not grow further. The CL visible sonographically before treatment

evidenced a reduction in size at the time of first examination at 4th day post treatment in 18 camels and in one camel at day 8, however the CL continued to be existent in one camel till day 20 of examination and there was no follicle growth in this camel.

5. Ovarian cysts

A high proportion (75%) of camels (n=6) with ovarian cysts evidenced cocking behaviour for prolonged periods (more than 2 months before September) and a high plasma progesterone (<1.5 ng/mL). Sonographically cysts showed hyperechogenic streaks in an anechogenic lumen in 75% (6/8) of the camels whereas in 25% camels (2/8) the ovarian cysts evidenced anechogenic structure with a thick echogenic wall. There was no evidence of pregnancy. Treatment of 8 camels resulted in disappearance of the cyst within 8 days and a mature follicle appeared in 5 camels' (62.5%) within 12 days of treatment. In 3 camels there was neither disappearance of cysts nor appearance of follicle during the study period.

6. Pregnancy rates

The pregnancy rates in different groups during the study period showed that a proportion of camels do evidence follicular activity during the transition phase (September-October) and can become pregnant when mated. Likewise treatments with both eCG and Ov-Synch protocols resulted in high pregnancy rates. Pregnancy rates were equally good in camels that were treated with prostaglandins and in camels treated for ovarian cysts (Table 1).

7. Efficiency of pregnancy diagnostic tests

Cocking of tail was evidenced by all (76) pregnant camels at 15-20 days of mating irrespective of whether they were treated or untreated. The cocking of tail continued till day 30 in all camels that were found pregnant at this time. Progesterone assay was accurate in determining the pregnant camels at day 30 irrespective of whether the camels were treated or untreated. Out of the total 90 camels mated in different treatment groups 56 camels were considered pregnant at day 20, and twenty camels were considered doubtful (plasma progesterone between 1-2 ng/mL) and 14 were considered non-pregnant. However at day 30, sixty nine camels were confirmed pregnant. Of the 20 doubtful camels 13 camels had elevated progesterone by day 30 and were confirmed pregnant, whereas in 7 camels the plasma progesterone declined below 1 ng/mL. Early embryonic death had occurred in these 7 camels (Table 2). In 3 of these 7 camels plasma progesterone was found greater than 2 ng/mL at day 20 and were confirmed pregnant at that time. The ultrasonographic appearance of fluid was found in all pregnant camels at day 20. At day 30 the conceptus fluid and embryo was visible in 80% of camels whereas only fluid was evident in 20% camels. There was disappearance

Table 1: Follicle appearance and pregnancy rates in camels treated with different hormones two months ahead of the natural breeding season.

S.No	Treatment	No of camels treated	Number of camels showing mature follicle	Day of appearance of mature follicle after treatment Day of treatment =Day 0	Pregnancy %
1.	Untreated control	30	8	-	4/8 (50.0%)
2.	eCG	43	34	8	28/43 (65.11%)
3.	Ov-Synch	39	28	12	24/39(61.53%)
4.	hCG (Ovarian cysts)	8	5	12	3/8 (37.50%)
5.	PG	20	15	8	10/20 (50%)
Total		140	90		69/90 (76.66%)

Table 2: Mean serum progesterone profiles (ng/mL) at day 20 and day 30 post mating in pregnant camels, non-pregnant camels and camels with early embryonic deaths.

	Day 20 (Mean ± SEM)	Day 30 (Mean ± SEM)
Non-pregnant camels (n=14)	0.28 ± 0.07	0.45 ± 0.08 NS
Pregnant camels (n=69)	2.37 ± 0.07	3.51 ± 0.12 NS
Camels with embryonic deaths (n=7)	1.67 ± 0.20	0.82 ± 0.05 NS

of fluid in camels that were found non-pregnant at day 30 by plasma progesterone.

Discussion

Mature ovarian follicles capable of ovulation on mating were evident only in a small proportion of camels during the two months before (September to October) the actual breeding season (November to April). A lower ovarian activity was recorded in camels during spring season (El-Harairy *et al*., 2010). Weekly ultrasound evaluation of camels during the month of September-October has shown small sized follicles only in one of the six camels but the follicle did not progress to the ovulatory size (Dholpuria *et al*., 2012). The differences could be because of differences in the environmental temperature, relative humidity and length of daylight as they play a major role in regulation of the seasonal ovarian activity in the female dromedary camels (El-Harairy *et al*., 2010). It is generally reflected in some studies that during the seasonal anestrus less number of follicles are observed

over the ovaries of camels (Abdoon, 2001 and Hussein *et al.*, 2008) however, a few studies depict the presence of ovulatory size follicles over camel ovaries which ovulate on mating with males (Vyas *et al*., 2008 and Dholpuria *et al.*, 2012) or spontaneously in a small proportion of camels (Nagy *et al*., 2005). In untreated control camels some follicular growth was evident on the periphery of the ovaries but clearly visible follicles (4-5 mm) or a mature follicle (1.0-2.0 cm) was visible only in a small proportion of camels. A recent study had shown similar findings in untreated camels that were monitored for 8 weeks during the months of September and October (Dholpuria *et al*., 2012). In another recent study it was shown that the season affects the growth of dominant follicle during its dominance period but the other characteristics of the follicular wave are not affected (Manjunatha *et al.*, 2012). It would thus be beneficial to monitor the camels during two months ahead of the breeding season and mate them if mature follicles are observed to obtain early pregnancies.

Equine chorionic gonadotropin (eCG) is the most widely used treatment for inducing ovarian follicle growth and estrus in camels including treatments during the non-breeding season (Elias *et al*., 1985; Dafalla *et al.*, 1987; Agarwal *et al*., 1993; Al-Sobayil, 2003 and Khalid and Al-Sobayil, 2008) with high success rates and evidence of ovulatory sized follicles on day 13 when eCG was used in succession to vaginal implants of progesterone releasing intra-vaginal device used for cattle (CIDR-B) (Khalid and Al-Sobayil, 2008 and Monaco *et al*., 2012). An earlier presence of an ovulatory sized follicle in camels in the present study could be because of the single use of eCG without prior progesterone treatments. Ovarian cysts that developed with doses of eCG (2000 IU) used in the present study could be because of a prolonged half life of 72 h seen with eCG, hence still lower doses are suggested but doses as low as 1000 IU given for 2 consecutive days after an progesterone injection resulted in ovulations in 50-75% camels only (Agarwal *et al.*, 1997) but none of the camel developed ovarian cyst. Thus, progesterone priming before eCG would be beneficial although this delays the appearance of ovulatory size mature follicle (Monaco *et al*., 2012).

The Ov-Synch protocol is widely used for timed insemination in cattle but less frequent for camel. Although the use of GnRH alone (Bono *et al*., 1991; Homeida *et al*., 1991; Skidmore *et al*., 1997; Ismail *et al*., 1998) or in combination with progesterone implants (Monaco *et al*., 2012). has been suggested for estrus and ovulation induction in camels however, the use of Ov-Synch protocol has not been documented. Mature follicles were visible on day 12 of the Ov-Synch protocol during the present study hence while using this protocol the presence of a mature follicle should be assured before mating instead of insemination or

AI on day 9 as suggested for cattle. In a previous study PG was injected 8 days after injection of 5000 IU of hCG (on palpation of mature follicles) and follicles could be palpated 3-5 days post PG injection (Ismail *et al*., 1998) during the breeding season. In the same study GnRH injections resulted in development of mature follicles after 6-8 days post injection during the non-breeding season. Similar findings were also recorded previously in another study (Bono *et al*., 1991). In a more recent study mature follicles appeared in camels treated with GnRH (Day 0) followed by a PG (Day 7) at 14 days from the start of treatment (Skidmore *et al*, 2009).

The presence of corpus luteum by sonography in 20 camels that failed to evidence tail cocking suggested previous spontaneous ovulation followed by a persistent CL or leutinization of unovulated haemorrhagic follicles (Shalash and Nawito, 1964 and Tibary and Anouassi, 2000). Spontaneous ovulations have been recorded in camels (Nagy *et al*., 2005).. The presence of CL on ovaries also indicated an early follicle growth and probably ovulation, leutinaztion of unovulated follicles or the persistence of a CL of the previous breeding season. Prostaglandin could easily regress the CL and mature follicles were seen on day 8 of treatment. Previous studies have shown that there is a sharp decline in progesterone after luteolysis in camel (Skidmore, 2005) and mature ovulatory sized follicles are present on the ovaries within 4 to 5 days of treatment (Ismail *et al*., 1998).

Ovarian cysts were observed in 5.71% (8/140) camels during the present study. The incidence of ovarian cysts has been recorded to vary from 0.9 to 14.0% during different seasons in camels (Musa, 1984; Al-Ani *et al*., 1992; Hegazy *et al*., 2004 and Ali *et al*., 2010) with higher incidence during spring and autumn months (Hegazy *et al*., 2004). Camels with ovarian cysts are increasingly being diagnosed using ultrasonography (Ali *et al*., 2010 and Vyas *et al*., 1998) and these cysts may persist up to 72 days in the absence of therapy (Ali *et al*., 2010). A deficiency of LH probably occurs because of an absence of a mating or poor release subsequent to mating (Tibary and Anouassi. 1996 and Abdel Rahim and El-Nazier, 19987). Camels with ovarian cysts evidenced cocking of the tail and progesterone values above 1.5 ng/mL during the present study. Skidmore (2000) had mentioned that cocking of the tail can be shown by camels given progesterone therapy. Gonadtrophin therapy (hCG) of camels with ovarian cysts was successful with 62.5% of camels evidencing an ovulatory size follicle along with cyst disappearance within 12 days of treatment. Previous studies have suggested the administration of GnRH (Vyas *et al*., 1998 and Skidmore *et al*., 1996) and hCG (Skidmore *et al*., 1996 and Agarwal *et al*., 1996) with good clinical outcome.

Pregnancy rates in camels evidencing follicle growth without any treatment were comparable to those in camels induced to estrus with eCG, Ov-Synch

or PG treatments. In a previous study (Vyas *et al*., 2008) the pregnancy rates were only 25% in camels detected with follicle and mated during July-August. In eCG treated camels similar pregnancy rates have been reported (Agarwal *et al*., 1996; Elias *et al*., 1985; Agarwal *et al*., 1993 and Khalid and Al-Sobayil, 2008). The use of Ov-Synch protocol has not been documented in camel hence comparison is not possible. The three parameters commonly used and studied for pregnancy diagnosis in camels in the present study showed close correlation. Cocking of the tail was fairly accurate in pregnancy diagnosis at day 15-20 of pregnancy. Although plasma progesterone values were accurate even at day 20 but due to early embryonic deaths that occurred after day 20, values at day 30 were more accurate for pregnancy diagnosis. Camels with early embryonic deaths evidenced the disappearance of the conceptus fluids and embryo instead of appearance of an echogenic embryo and increasing fluids observed in camels with normal progression of pregnancy. Camels with embryonic deaths between day 20 and 30 evidenced a significant decline in the plasma progesterone values. Concomitantly camels also stopped showing tail cocking. Plasma progesterone concentrations recorded in pregnant camels vary from1.40 to 6.45 ng/mL (Abdel Rahim and El-Nazier, 1987 ; Agarwal et al., 1987; Agarwal et al., 1997 and Skidmore, 2000). Plasma progesterone increases to 1.0 ng/mL four days post hCG injection and reach its maximum on day 8 post injection El-Wishy *et al*., 1983 and Ismail *et al*, 1998). Although camels with progesterone values above 1 ng/mL at day 20 post mating are considered pregnant (El-Wishy *et al*., 1983; Elias et al., 1984) Dholpuria et al., 2012) we considered camels with progesterone values between 1-2 ng/mL as doubtful because of the possibility of early embryonic deaths as also recorded previously (Dholpuria *et al*., 2012). The proportion of early embryonic losses during the present study was 9.21%. Previous studies had recorded embryonic losses in camels ranging between 5.7 to 16.9% in primiparous and multiparous camels (Al-Juboori and Baker 2012 and Pratap *et al*., 2012). It has been mentioned that 10-15% of camels diagnosed pregnant at 20 days are found to have lost the embryo by day 40 (Khan *et al.,* 2003). The causes of embryonic losses in camels have been described to be of infectious origin (Tibary *et al*., 2006) and low progesterone or hostile uterine environment (Ali *et al*., 2010 and Pratap *et al*., 2012) however, the causes of embryonic losses were not ascertained in the present study.

It was concluded that a small proportion of camels evidence a mature follicle between September and October months and follicle growth and conception can be stimulated two months ahead of the natural breeding season in camels by the use of either eCG or Ov-Synch treatments. Camels evidencing a persistent CL or ovarian cysts should be treated with PG or hCG for obtaining

follicle growth and pregnancy. Camels evidencing serum progesterone profiles above 2.0 and 3.0 ng/mL at 20 and 30 day of mating should be considered pregnant.

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