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Problems of artificial insemination in dromedarius camel - failure of ovulation and entrapment of spermatozoa in gelatinous camel semen

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ABSTRACT

An artificial insemination study was conducted on 17 female camels which were administered human Chorionic Gonadotrophin (hCG) to induce ovulation after confirming a follicle in the ovaries using sonography. The animals were inseminated with either diluted-cooled or fresh undiluted semen. No female camel could be impregnated with diluted and cooled semen, while pregnancy rate was low with neat undiluted semen. To ascertain possible causes of low conception rate, plasma progesterone (P_4) profiles were monitored. Criteria adopted for interpretation of these profiles were as follows: P_4 levels below 1 ng/ml on days 5-8 was considered to indicate failure to ovulate; a single peak of 1ng/ml on days 5-8 followed by a decline on day 12 was considered to indicate ovulation. However, failure of fertilization and P_4 levels of more than 1 ng/ml on days 5-8 and day 12 followed by a decline was considered to indicate successful ovulation and fertilization, but failure of embryo survival. Consistently higher levels of P_4 were considered to be indicative of pregnancy. Using these criteria, 5 of 33 inseminations were diagnosed as pregnant, while profiles of 17 of 33, 8 of 33 and 3 of 33 were indicative of failure of ovulation, failure of fertilization and failure of embryo survival, respectively. A high incidence of failure of ovulation may be due to oversized follicles or follicles in which degenerative processes might have been initiated prior to administration of hCG. High failure of fertilization may be due to a viscous form of camel semen, which may play a role as a sperm reservoir and protect the viability of spermatozoa in the female genital tract by entrapping sperm. Insemination with diluted and cooled semen may disturb the protection, resulting in failure of conception. It is concluded that the high incidence of ovulation failure and failure to deposit sperm in its natural entrapped viscous form are the major problems for development of AI in the camel. Further improvement may be expected, if we are able to standardize the appropriate insemination time around peri ovulatory time, and appropriate follicular size, which responds to hCG.

Key words: *Camelus dromedarius*, artificial insemination, ovulation

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Introduction

Artificial insemination (AI) is a well accepted method used to achieve faster genetic improvement in livestock species. This has been developed to its optimum extent in cattle and buffalo, while in some other domesticated species of livestock, including the camel, it is yet to be developed. AI is claimed to have been highly successful in Bactrian camels (ARTHUR, 1992), but in dromedary camels results have been less encouraging. Workers at the Camel Reproduction Centre, Dubai have claimed pregnancy rates of 50-60% with fresh diluted semen used within 30 minutes of collection, but conception rate decreased dramatically to 25-30% if semen was not used for 24 hours. All the pregnancies were established with a particular extender and no pregnancy could be established with frozen thawed semen (SKIDMORE, 2003). Likewise, pregnancy results from almost nil with diluted-chilled/frozen-thawed semen to 40% with whole semen have been reported in India (DEEN et al., 2003). The reasons for poor success have not been determined but a major difficulty with AI in camel is to ensure ovulation in inseminated animals (ARTHUR, 1992). ANOUASSI et al. (1992) also observed that the incidence of ovulation and pregnancy is significantly lower in female camels inseminated either with fresh undiluted or diluted semen alone than those obtained with AI following mating with vasectomised teaser. MUSA et al. (1992) opined that deposition of 1 ml of fresh semen, or else exogenous administration of hCG, is essential to ensure ovulation. Entrapment of spermatozoa in thick viscid camel semen (BROWN, 2000) and its speculated role as a sperm reservoir in the female genital tract also need to be considered. *In vitro* extension of semen and its storage could hinder the protective role of this natural reservoir to the spermatozoa in female genital tract.

Camel is an induced ovulator and it has a short luteal phase in non-pregnant female camels (AGARWAL et al., 1991). ARTHUR (1992) reported that unmated and anovulatory camels have basal progesterone levels, those which ovulate but do not conceive show a peak value of 6-10 days after mating, which declines to baseline by day 12. A high progesterone concentration beyond day 12 after mating is a very strong indication of pregnancy. Thus, peripheral progesterone profiles of individual female camels after AI may enable the detection of ovulation, fertilization and pregnancy, which in turn may provide an insight into the possible causes of low success of AI in this species and pave the way for further courses of research to strengthen this technique. With this objective in mind progesterone profiles of inseminated female camels were monitored to assess status of ovulation, fertilization and embryo survival in these females.

Materials and methods

Thirty-three female camels proposed for experimental artificial insemination were subjected to routine screening for ovarian follicles using pie scanner-200 ultrasound machine and trans-vaginal transducer of 5 MHZ capacity (Pie Medical equipment B.V.

Philipsweg, The Netherlands) The camels were sedated with 100 mg of xylazine (Izine^R, Intaas Pharmaceuticals, India) administered intravenously while restrained in a sitting posture. Those females which exhibited mature ovarian follicle of 15-20 mm or greater in diameter were injected intramuscularly with 5000 IU of hCG (Profassi^R, Serono, Italy). The animals were inseminated 24-48 hrs after injection either with cooled semen, frozen thawed semen or with whole semen. Tris egg yolk (FOOTE, 1970) glycerol was used to extend the semen. Semen was extended initially at a rate of 1:1 with unglycerolated diluent and then a final dilution to 1:3 with glycerolated dilutor within 30 min of collections. The semen samples were packaged after extension into labelled plastic cryovials of 2 ml capacity, which were placed in a glass beaker containing water (25 °C) for cooling in a refrigerated unit to 4 °C within 2 hrs. They were then further maintained at this temperature for another 3 hrs.

Freezing of semen was carried out in an automated liquid nitrogen based programmable freezer KRYO 10-1.3 (Planer Products Limited, U. K.) using the protocol of ALMQUIST (1969) developed for dairy cattle, which is as follows:

Start temperature	4 °C
Cooling Rates	
From 4 °C to -15 °C	-1 °C per min
From -15 °C to -60 °C	-4 °C per min
From -60 °C to -100 °C	-20 °C per min

Finally, the cryovials were plunged and stored in liquid nitrogen until required.

Thawing was accomplished by immersing the vial containing frozen semen in a water bath at 40 °C for 2 minutes. Post-thaw motility examinations were accomplished using thermostatic stage inverted phase contrast microscope (Nikon).

Artificial insemination. The female camels were restrained in a sitting position and sedated with xylazine 100 mg i v (Izine®, Intaas Pharmaceuticals, India). For inseminations using diluted semen, a hard plastic catheter was passed per vagina to the cervix and then manipulated by recto-genital palpation into the uterus. Six ml of semen (1:3 extension) was inserted through the pipette into the uterus with a syringe. For whole semen (3-5 ml) deposition, a 20 ml syringe was used. Blood samples were collected by jugular venepuncture in heparinized vials on days 0, 3, 7, 10, 14, 17, 21, 24, 28 and plasma was separated in a refrigerated centrifuge (C- 24, Remi, India) at 2500 rpm. Plasma samples were preserved at -20 °C until used for P_4 analysis. Progesterone analysis of plasma samples was conducted with Coat- A- Count RIA kits (PITK PG-1, 2002-12-11) of Diagnostic Products Corporation, Los Angeles, CA 90045-5597. Inter- and intra-assay coefficients of variations were 5 and 7.5%, respectively, and minimum detection limit was 0.1 ng/ml.

Ovulation, fertilization and pregnancy were determined through progesterone profiles using the following criteria: anovulatory camels had basal progesterone levels (less than

1 ng/ml), while those that ovulate, but do not conceive, exhibit peak values (more than 1 ng/ml) at 6 to 10 days after mating, which then declines to baseline by day 12. Those camels which ovulate and conceive show P_4 values >1 ng/ml on both days 6-10 and day 12. Early embryo loss was suspected when progesterone profiles declined to basal levels after day 12, while pregnant camels show elevated progesterone concentrations throughout gestation and were also confirmed to be pregnant by rectal palpation.

Mean ± SE on progesterone profiles on different days were calculated for each group, and chi-square test was applied.

Results

Results are presented in Table 1 and Fig. 1 in the form of a line diagram. Fig.1 shows mean progesterone profiles of all 4 groups of female camels. Series 1 comprised mean progesterone values indicative of luteal phase. All these animals were confirmed clinically pregnant, completed their gestation and delivered healthy calves. Series 2 comprised a mean progesterone profile of 17 female camels, which exhibited levels below 1 ng/ml, indicating failure of ovulation. Series 3 comprised progesterone profiles of 8 female camels, which showed only two points above 1 ng/ml. These levels indicated ovulation but failure of fertilization. Series 4 comprised mean progesterone profiles of 3 female camels, which showed higher profiles at more than 2 points, followed by a decline. These levels indicated successful ovulation and fertilization, but failure of embryo survival. Based on these results, the low conception rate in AI in camel can be categorized and rated as follows:

Failure of ovulation 17/33 (51.51%)
Failure of fertilization 8/33 (24.24%) and
Early embryonic death 3/8.

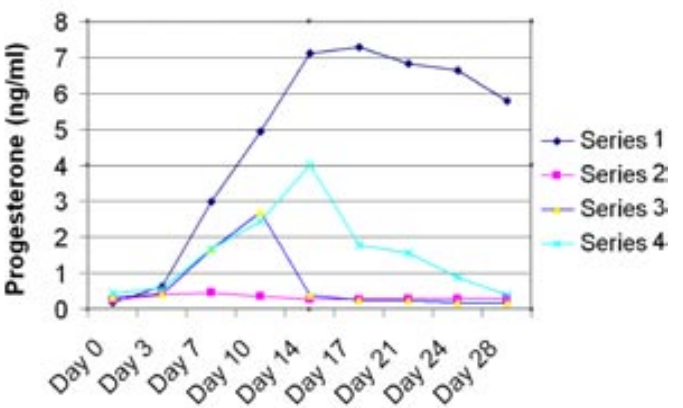

Fig.1. Progesterone profiles of artificially inseminated female camels

Table 1. Mean ± SE of progesterone profiles of artificially inseminated female camels grouped as pregnant, failure to ovulate, failure to fertilize, and early embryonic deaths

Group	Days, progesterone (ng/ml)								
	0	3	7	10	14	17	21	24	28
Chi-square value significance	-	-	ns	s	s	s	s	s	s
Pregnant	0.19 ± 0.06	0.63 ± 0.18	2.99 ± 0.78	4.94 ± 0.57	7.12 ± 1.48	7.28 ± 1.25	6.83 ± 1.72	6.64 ± 1.32	5.80 ± 1.21
Failure to ovulate	0.25 ± 0.04	0.4 ± 0.06	0.46 ± 0.06	0.35 ± 0.05	0.28 ± 0.03	0.27 ± 0.03	0.29 ± 0.04	0.29 ± 0.05	0.29 ± 0.03
Failure to fertilize	0.32 ± 0.12	0.42 ± 0.07	1.66 ± 0.52	2.70 ± 0.75	0.39 ± 0.08	0.26 ± 0.05	0.23 ± 0.04	0.19 ± 0.05	0.17 ± 0.05
Early embryonic death	0.44 ± 0.19	0.58 ± 0.13	1.69 ± 0.72	2.45 ± 0.64	4.01 ± 1.51	1.78 ± 1.36	1.57 ± 1.44	0.89 ± 0.54	0.39 ± 0.10

ns - nonsignificant, s - significant

Chi-square test on mean values of peripheral plasma progesterone profiles indicated significant differences in four groups of camels, viz. pregnant, failure to ovulate, failure to fertilize and early embryonic deaths.

Discussion

Since the female camel is an induced ovulator and ovulates only after mating with a male camel, it becomes essential to induce ovulation in AI program by exogenous administration of hormones (MUSA et al., 1992) or by mating with vasectomised teaser (ANOUASSI et al., 1992). Mating with a teaser is considered not to be practical due to the potential spread of venereal diseases. Exogenous administration of hCG or GnRH is preferred to induce ovulation. However, despite exogenous administration of hCG, success rates are not high, as observed in the present study, where 51.51 % female camels did not ovulate. Similar problems of ovulation in inseminated female camels were reported by ANOUASSI et al. (1992), who observed ovulation in only 33 and 20% of female camels, which were inseminated with raw or diluted semen, respectively, as compared to 60% in those mated with a vasectomised teaser. This problem of anovulation needs to be resolved if AI is to be developed in this species. The size of the follicle at the time of administration of hCG and AI is reported to play an important role. In general, 83-85% of the females were reported to ovulate after breeding if the diameter of the existing follicle was between 13 and 16 mm. Ovulatory response to treatment with hCG, GnRH or mating was reported to be highest when the size of the dominant follicle was between

10 and 22 mm, while follicles that were larger than 22 mm might have already begun a degeneration and may not respond to treatment (ANOUASSI et al., 1994). SKIDMORE et al. (1995) also reported as many as 35-45% to 50% anovulatory follicles. It is possible that poor ovulation response in the present study could have been due to oversized follicles which might have begun degenerative changes prior to treatment. Thus, improvement in efficiency can be possible by selecting appropriately sized follicles prior to treatment. Additionally, exact mechanism of ovulation in this species also needs further investigation. Chinese workers in particular held the view that a GnRH-like hormonal factor is present in seminal plasma and initiates ovulation (ZHAO, 2001), while, MUSA and ABUSINEIA (1978) and SHELDRICK et al. (1992), could find no such evidence of injection of whole semen, seminal plasma, water or prostaglandin in the release of sufficient LH from the pituitary gland to cause ovulation. Mechanical stimulation of the cervix has also been found not to hasten ovulation in the camel (MUSA et al., 1990). As such, the ovulatory response in the camel could be the result of a combination of stimuli, including a chemical factor in the seminal plasma, neuro humoral response to the mechanical stimulation of the coitus, and the male effect (MARIE and ANOUASSI, 1987; MOSLAH et al., 1992). Ovulation and pregnancy rates were significantly higher in inseminated camels which had been mated by a vasectomised male (ANOUASSI et al., 1992). Further investigations on the role of the male in inducing ovulation, particularly poll gland secretion, may be helpful in devising appropriate measures to improve ovulation efficiency.

In the present study, the pregnancy rate with refrigerated diluted semen was nil. Successful impregnation of female camels could be possible with deposition of whole semen only. Successful pregnancy with frozen thawed semen was reported only in Bactrian camel by Chinese workers (CHEN et al., 1990; ZHAO et al., 1990). In fact, they reported pregnancy rates of 87-91%, which is much higher than would be expected, even from natural mating. Moreover, no repetition of such successful application in other parts of the world has been reported. For example, no pregnancies have been reported to date after inseminating the dromedary camel with frozen semen (SKIDMORE, 2003); pregnancy rates of 50-60% have been reported in camels inseminated with fresh diluted semen within 30 minutes of collection (ANOUASSI et al., 1992). Conception rate decreased to 25-30% in camels inseminated with semen cooled and stored for 24 hr. Moreover, all the pregnancies were achieved with cooled semen diluted in Green buffer (IMV) + 20% egg yolk only. CALDERON et al. (1968) also observed very poor pregnancy rates in small camelids, which could be improved using whole undiluted semen (BRAVO et al., 1997). Successful impregnation of female camels with undiluted semen, and no success with diluted chilled semen, indicate some important seminal factor. Camel semen was formerly used in gel form, and spermatozoa are entrapped in gel. Entrapment seems to play a vital role similar to that of a sperm reservoir in other species. Dilution of semen with extenders and in vitro storage might disturb the reservoir form of function and protective action of semen gel on

sperm viability, which may be responsible for failure of impregnation with frozen thawed and cooled- stored semen.

Conclusion

It is concluded that failure of ovulation has been the major factor responsible for low conception rates in artificial insemination in the present study. Size of follicle needs to be monitored prior to administration of hCG. Those greater than 18-20 mm must be avoided in order to improve ovulation rate. Another important factor, which must be considered, is the viscosity and gel form of semen, which seems to serve as a sperm reservoir in the female genital tract and has some protective action on the viability of spermatozoa. These problems need to be resolved if AI has to be developed in this species.

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SAŽETAK

Umjetno osjemenjivanje provedeno je na 17 deva kojima je dan ljudski korionski gonadotropin (hCG) u svrhu poticanja ovulacije. Prethodno je ultrazvučnom metodom ustanovljen folikul na jajnicima pretraženih deva. Deve su osjemenjene ili razrijeđenom i rashlađenom ili svježom, nerazrijeđenom spermom. Nijedna deva nije bila oplođena nakon osjemenjivanja razrijeđenom i rashlađenom spermom, a nakon osjemenjivanja nerazrijeđenom spermom stopa oplođenosti bila je niska. Radi utvrđivanja mogućih uzroka niske stope koncepcije, utvrđivana je razina progesterona u plazmi (P_4). Razina P_4 ispod 1 ng/ml u razdoblju 5 do 8 dana smatrala se pokazateljem izostanka ovulacije. Jednokratni porast od 1 ng/ml tijekom 5-8 dana nakon kojeg je slijedio pad 12. dana, smatrao se pokazateljem ovulacije s neuspjelom oplodnjom. P_4 razine veće od 1 ng/ml tijekom razdoblja 5 do 8 dana, uključujući 12. dan nakon kojeg slijedi opadanje smatrale su se uspješnom ovulacijom i oplodnjom ali neuspjelim preživljavanjem embrija. Postojano visoke razine P_4 smatrale su se pokazateljem gravidnosti. Prema navedenim kriterijima, pet od 33 osjemenjivanja rezultirala su gravidnošću. U 17 od 33 osjemenjivanja izostala je ovulacija. U 8 od 33 osjemenjivanja nije došlo do oplodnje, a u tri od 33 osjemenjnivanja embrij nije preživio. Učestala pojavnost izostanka ovulacije mogla bi se povezati s prevelikim folikulima ili folikulima u kojih su degenerativni procesi započeli prije davanja hCG. Visoka učestalost neuspjele oplodnje, mogla bi se povezati s viskoznošću sperme, zbog koje su spermiji u genitalnom traktu deve zaštićeniji i lakše preživljavaju. Osjemenjivanje s razrijeđenom i rashlađenom spermom može poremetiti navedene zaštitne mehanizme i dovesti do slabije oplođenosti. Može se zaključiti da je učestalo izostajanje ovulacije i nemogućnost polaganja sperme u njezinom prirodno viskoznom obliku glavni problem za razvoj umjetnog osjemenjivanja deva. Daljnji napredak se može očekivati ako se standardizira prikladno vrijeme osjemenjivanja (periovulatorno vrijeme), te utvrdi veličina folikula koja najbolje odgovara na aplikaciju hCG.

Ključne riječi: jednogrba deva, umjetno osjemenjivanje, ovulacija