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IS LOW EFFICIENCY UNDER AI IN CAMEL DUE TO OVULATION PROBLEMS?

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ABSTRACT

The study was conducted on 10 breedable female camels of 6 to 10 years of age bearing mature sized follicles in their ovaries as revealed by sonographic examinations (Pie-scanner-200 using transvaginal transducer of 7.5 MHz capacity). Exogenous hormone (Profassi, 5000 i.u., Serono, Italy) was administered to induce ovulation followed by artificial insemination. The objectives of the study were to evaluate efficiency of hCG to induce ovulation and to impregnate female camels with artificial insemination (AI). Peripheral plasma progesterone analysis either daily or on alternate days from day 0 to 30 was used to assess ovulation and pregnancy status. Behavioural and clinical examinations were also regularly performed for pregnancy. Blood samples for analysis of peripheral plasma progesterone were regularly harvested and quantified by RIA kits®. Counting of radioactive disintegration was accomplished in automated gamma-counter PC-RIA MAS 06. None of the 10 inseminated female camels conceived with 0.5 to 1.0 ml of freshly collected camel semen deposited into the uterus. Nine out of 10 female camels exhibited a significant rise in P_4 (>1ng/ml) at varying stages after hCG administration barring one. The first rise in P_4 concentration above 1ng/ml after hCG administration was recorded on days 3, 4, 6, 8, 9 and 10 in 1, 3, 2, 1, 1 and 1 of the 9 responding females, respectively. P_4 concentration above 1ng/ml persisted for 2-3 days in 5/9 females and 5-7 days in 4/9 females. The magnitude of rise in P_4 concentration was greater in later as compared to former group. The P_4 profiles of later group of animals indicated that these animals have definitely ovulated and developed a normal corpus luteum, while P_4 profiles of another 5 females are difficult to interpret in terms of ovulation and corpus luteum formation. Either these animals have undergone follicular leutinisation without ovulation or else ovulated and developed a weak corpus luteum with short life span. It is concluded that 40% of hCG treated and inseminated female camels have definitely ovulated and developed a normal corpus luteum. More work is required to assess the failure of conception in these females, which apparently may be due to low dose of inseminated semen. It is difficult to interpret the induction of ovulation in another 5 females because of relatively low magnitude and duration of progesterone rise.

Key words: AI, hCG, Ovulation, Progesterone, RIA

Artificial insemination in dromedary camel could not be developed to an accepted level despite average results reported from Camel Reproduction Centre, Dubai and remarkable success in bactrian camel from China (Zhao, 2000). Results reported from Camel Reproduction Centre, Dubai indicated pregnancy rates of 50-60% with fresh semen used with in 30 minutes of its collection, the conception rate dramatically decreased to 25-30% with diluted semen cooled for 24 hours. Although post thaw motility has looked promising, no pregnancies could be obtained after inseminating dromedary camel with frozen semen (Skidmore, 2003). Contrary to this, remarkable success rate of 80-100% was reported in bactrian camels with frozen thawed semen from China (Zhao, 2000). But, such results have not been obtained for dromedary camels at National Research Centre on Camels (NRCC) either with chilled or frozen thawed semen. At NRCC, successful artificial impregnation of female camels could be achieved only by depositing neat semen (Aminu Deen *et al*, 2003). Similarly, no pregnancies were achieved using transcervical intrauterine AI with chilled or frozen thawed semen in alpacas (Voughan *et al*, 2003). Camel is an induced ovulator. Ovulation and formation of corpus luteum occurs only after mating. Induction of ovulation is a pre-requisite to artificial insemination either with mating to a vasectomised camel or through exogenous administration of hormones (Anouassi *et al*, 1992). A major difficulty with camel artificial insemination is to ensure that the inseminated animals ovulate (Arthur, 1992).

The objectives of the study were to evaluate efficiency of hCG to induce ovulation and to impregnate female camels with artificial insemination.

Materials and Methods

This study was conducted on 10 breedable female camels selected by sonographic examinations for mature sized follicles in their ovaries. The animals were sedated with xylazine (Izine[â], Intaas

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Pharmaceuticals, 100 mg injected intravenously) and restrained in sitting position by using cotton ropes. Sonographic examinations were accomplished with Pie-scanner-200 using transvaginal transducer of 7.5 MHz capacity. The animals with follicular diameter measuring 1-2 cm were selected for study.

HCG 5000 I.U. (Profassi,â Serono, Italy) was administered in the selected animals (day 0) followed by insemination 48 hr later with 0.5-1.0 ml whole semen deposited into the uterus with bovine insemination gun and ½ cc straw. Straws were filled by aspiration pump with fresh neat camel semen collected by AV method. The filled straws were then loaded in an AI gun. The straws were adhered to AI gun with adhesive bandage. Plastic sheaths used in bovine AI were not applied. For artificial insemination, females were sedated and restrained, and AI gun was traversed through vagina into the cervix by vaginal manipulation and then to uterus through rectogenital palpation. Semen was deposited into the uterus. Behavioural and clinical examinations for diagnosis of pregnancy were conducted at appropriate times.

Peripheral plasma progesterone profiles were monitored from day 1 to day 30, either daily or on alternate days to evaluate efficiency of hCG to induce ovulation. Blood samples were harvested daily from day 1 to day 30 by jugular vene-puncture in heparinised vials. Plasma was separated in refrigerated centrifuge (C-23, REMI) and preserved at –20°C till used for assay. Progesterone was quantitated by RIA kits from IMMUNOTECH SAS, France.

Diverse peripheral plasma progesterone concentration(P_4) was suggestive of ovulation and normal corpus luteum formation (Above 1 ng/ml level with continually increasing trend for 5-7 days), leutinisation of follicle without ovulation (1-2 ng/ml level for 3-5 days), failure of ovulation and leutinisation (Below 1 ng/ml), pregnant (Above 1 ng/ml level without decline) and non-pregnant (declining to basal level).

Results

None of the 10 inseminated female camels conceived with 0.5 to 1.0 ml of freshly collected camel semen deposited into the uterus. This was evident with behavioral (tail cocking), clinical (recto-genital palpation) and endocrine investigations. One of the major factors speculated for failure of conception is uncertainty of ovulation in female camels in absence of copulation with male. Attempts were made to investigate this factor with peripheral plasma P_4 profiles monitored daily or on alternate days from day 0 (day of administration of hCG) to day 30.

Peripheral progesterone profiles are presented in table 1. Nine out of 10 female camels exhibited a significant rise (>1 ng/ml) at varying stages after hCG administration, while one appear to have not responded. The first rise in P_4 concentration above 1ng/ml after hCG administration was recorded on days 3, 4, 6, 8, 9 and 10 in 1, 3, 2, 1, 1 and 1 of the 9 responding females, respectively. P_4 concentration above 1ng/ml persisted for 2-3 days in 5/9 and 5-7 days in 4/9 females. The magnitude of rise in P_4 concentration was greater in later as compared to former group. The P_4 profiles of later group of animals indicated that these animals have definitely ovulated and developed a normal corpus luteum, while P_4 profiles of another 5 females are difficult to interpret in terms of ovulation and corpus luteum formation. Either these animals have undergone follicular leutinisation without ovulation or else ovulated and developed a weak corpus luteum with short life span.

Discussion

Results indicated that artificial impregnation was not possible with 0.5 to 1ml of freshly collected camel semen. Vaughan *et al* (2003) also reported no pregnancy using transcervical intrauterine AI with chilled or frozen thawed semen in alpaca. Other results reported previously, including our own are not at par with present study. Skidmore (2003) reported pregnancy rate of 50- 60% in insemination with fresh camel semen used with in 30 minutes of its collection but the pregnancy rate declined to 25-30% with chilled semen used 24 hrs after collection. It was further observed that pregnancy could be achieved only with green buffer diluted semen. However, no pregnancy could be induced in inseminations using frozen-thawed semen. In our previous experiment, we could not impregnate females with chilled semen but four females could be successfully impregnated with deposition of full volume of ejaculate without diluting. Remarkable success was reported by Zhao (2000) with frozen semen in bactrian camel, but similar results were not reported from anywhere else. In light of these facts, it appears probable that 0.5-1.0 ml semen may not be sufficient for conception. As suggested by Arthur (1992), more work is required to determine the minimum required amount of natural semen in the inseminating dose.

Another major difficulty with camel artificial insemination is to ensure that the inseminated animals ovulate. Anouassi *et al* (1992) reported that acceptable conception rates following insemination in the camel can only be achieved if ovulation is achieved reliably. Whether service by vasectomised male can regularly induce ovulation and how reliable hormone

Table 1. Progesterone profiles of hCG treated and artificially inseminated female camels over 30 days.

Camel No.	Days/ Progesterone (ng/ml)															
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
345	0.26	-	0.39	-	0.27	-	0.51	-	**1.21**	-	**1.68**	-	0.59	-	0.07	-
117	0.03	-	0.50	-	**1.26**	-	**1.89**	-	0.26	-	0.11	-	0.02	-	0.06	-
495	0.27	-	0.33	0.57	**1.11**	0.91	**1.58**	0.31	0.38	0.26	0.30	0.07	0.52	-	0.26	-
465	0.39	-	0.38	-	0.37	-	0.29	-	0.93	**1.08**	**1.44**	**1.02**	0.48	-	0.30	-
489	0.21	-	0.23	-	0.72	0.96	**1.43**	**2.48**	0.55	-	0.55	-	0.00	-	0.02	0.17
479	0.46	-	0.60	-	0.89	-	**2.21**	**-**	**5.02**	**-**	**2.37**	-	0.57	-	0.42	-
493	0.35	-	0.27	0.26	**1.51**	**2.31**	**4.08**	**3.32**	**4.80**	**1.42**	0.81	-	0.15	-	0.46	-
121	0.03	-	0.17	-	0.10	0.07	0.02	0.19	0.69	-	**5.14**	**8.32**	**20.54**	**13.51**	**19.05**	-
405	0.57	-	0.78	**1.07**	**2.67**	**3.32**	**8.26**	**8.90**	**12.74**	**1.77**	0.88	-	0.42	-	0.41	-
439	0.46	-	0.66	-	0.67	-	0.41	-	0.27	-	0.31	-	0.22	-	0.33	-
Camel No.	**Days/ Progesterone (ng/ml)**															
	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	
345	0.12	-	0.10	-	0.85	-	0.25	-	0.29	-	0.18	-	0.29		-	
117	0.11	-	0.04	-	0.08	-	0.10	-	0.23	-	0.11	-	0.15	0.13	-	
495	0.20	-	0.60	-	0.31	-	0.50	-	2.80	-	0.22	-	0.31	-	-	
465	0.10	-	0.24	-	0.18	-	3.54	-	0.86	-	0.68	-	0.27	-	0.37	
489	1.60	0.17	0.00	-	0.13	-	0.20	-	0.39	-	0.14	-	0.45	-	-	
479	0.36	-	0.50	-	0.31	-	0.37	-	0.42	-	0.39	-	0.27	-	0.37	
493	0.38	-	0.14	-	0.47	-	0.83	-	0.34	-	0.29	-	1.84	-	-	
121	0.27	-	0.15	-	0.02	-	0.03	-	0.15	-	0.06	-	0.10	0.14	-	
405	0.52	-	0.25	-	0.30	-	0.25	-	0.49	-	0.32	-	0.66	-	-	
439	0.08	-	0.46	-	1.23	-	0.29	-	2.03	0.24	0.36	-	0.28	-	-	

Bold values are above 1 ng/ml of early days

preparations like human chorionic gonadotrophin and GnRH, are for stimulating ovulation is required to be worked out. Progesterone profile results obtained in present study indicated that four out of ten hCG treated female camels have definitely ovulated and developed a normal functional corpus luteum. Since the magnitude of rise of progesterone and its duration is low in another five females, it is not possible to interpret as to whether these have ovulated and developed a corpus luteum or simply developed a leutinized follicle without ovulating.

In light of these findings, it is apparent that hCG treatment can induce ovulation in female camels if administered at appropriate time. It is concluded that 40% of hCG treated and inseminated female camels have definitely ovulated and developed a normal corpus luteum. More work is required to assess the failure of conception in these females, which apparently may be due to low dose of inseminated semen. It is difficult to interpret the induction of ovulation in another 5 females because of relatively low magnitude and duration of progesterone rise. More work is required to assess such cases with techniques like sonography.

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Book Review

Title : Diagnostic Parasitology
Authors : Veer Singh Rathore & Yogesh Singh Sengar
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Veterinary (and Medical) Parasitology has come a long way as a major discipline. Several areas within the broad field have evolved and grown so much in recent decades as to become sub-disciplines justifying the need for books/handbooks exclusively devoted to each of them. In the updated VCI dispensation for the syllabus of veterinary parasitology, parasitic techniques (diagnostic parasitology) has been granted its rightful independent status alongside, and at par with, such entities as parasitic zoonoses, parasitic pathology, parasitic immunity and immunoprophylaxis. So much new information has been generated in the diagnostics of parasites and parasitoses of late, that the need for a concise guidebook combining the material in a single source was being increasingly felt. There is a no gainsaying the importance of correct and timely diagnosis as the foundation of effective treatment and control. As such, the initiative of the authors in coming out with a companion volume to their earlier valuable contribution on "Parasitic Zoonoses" (published early 2005) is highly welcome.

The compilation is arranged in 14 chapters covering a broad spectrum of diagnostic materials and procedures, supported by 21 figures and 14 tables. The chapter on examination of faeces is comprehensive (41 pages) incorporating procedures related to even relatively emerging entities such as *Cryptosporidium* spp, apart from simple sketches of gastrointestinal protozoa and eggs of worm parasites of humans, different livestock species, dogs and poultry. The chapter entitled "Faecal Culture" is highlighted by tabulated data on the identifying features of larvae (L_3) of mainly gastrointestinal nematodes of different livestock species as well as a useful key for the identification of larvae in human coprocultures. However, culture media for protozoans such as *Leishmania* and *Trypanosoma* are incongruous under this chapter heading. The next chapter (Ch.3), "Preparation of parasites for examination" also includes collection and preservation which is, in effect, a partial overlap of information contained in a later chapter (Ch.8, Necropsy). A major positive feature of this chapter is a guide (key) to identify adult gastrointestinal nematodes of ruminants. Examination of body fluids other than blood, as diagnostic material for some helminthic eggs, has found coverage in a separate small chapter (Ch.5) following a bigger one on "Examination of Blood" (Ch.4). It is heartening to find full justice done to "Diagnostic methods in arthropods" (Ch.6) – an oft-neglected area, by including tabled identifying features and pictorial key for mange, itch and scab mites. Pathological aspects of diagnostic parasitology have been well addressed in the chapters 8 and 9. Necropsy (Ch.8) details the post-mortem lesions and the material to be submitted in different diseases. Under the title "Preparation of tissues for parasitic examination" (Ch.9), one can find essential information on impression (organ) smears, sectioning and preservation of tissues, in addition to histopathology and histochemistry of commonly prevalent parasitic diseases. The separate mini-chapters on Micrometry (Ch.10) and Scale drawing and Camera Lucida (Ch.11) could perhaps have been better combined under the heading "Microscopy", enlarged to include something regarding phase-contrast. Chapters on Immunodiagnosis (Ch.12), and Molecular Diagnosis (Ch.13) incorporate the latest advances such as monoclonal antibodies, radio-immunoassays, immuno-peroxidase, immuno-blotting, nucleic acid probes and PCR. Diagnostic adjuncts (Ch.14) should include mention of lab animal inoculation (for diagnosis of trypanosomosis) and tissue culture (for theileriosis).

The language is lucid and precise. The handbook will be useful guide for students and diagnosticians, as well as valuable reference for teachers and researchers, both Veterinary and Medical.

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