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## **Pregnancy Diagnosis in Camels** (17 June 2000)

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### **Introduction**

In managing any camelid herd efficiently there is a definite need to diagnose pregnancy as accurately and as soon as possible after mating so that if the camel is not pregnant she can be re-mated, re-inseminated or returned to an embryo transfer programme. There are several methods used to diagnosis pregnancy but it must be remembered that whatever method is used, a single pregnancy diagnosis is not sufficient to guarantee a birth, especially if done at a very early stage (i.e. before 40 - 50 days post mating). This is due in part to errors in diagnosis, but is also due to the high incidence of early embryo loss seen in these species. Further examinations should therefore be carried out at 3 - 4 months of gestation to ensure the pregnancy is developing normally.

### **Methods of pregnancy diagnosis**

#### Tail "Cocking"

Several Bedouin have asserted that it is possible to detect pregnancy in camels from as early as 15 days by observing an erect and coiled tail in the pregnant animal when approached by a male camel [1,2]. This is true, however, this response has been noted in unmated animals treated with exogenous progesterone and also in younger animals that maybe alarmed by the male.

#### Changes in Cervical Mucous

i) Viscosity - Other studies have shown that changes occur in pH and flow elasticity of the cervical mucous in pregnant versus non-pregnant camels. The cervical mucous tends to be turbid in most stages of the ovarian cycle, although during oestrus it becomes less viscous, but not watery. In pregnant females the mucous becomes whitish and opaque and it decreases gradually in amount until the second month when it becomes almost impossible to collect [3].

ii) pH - The pH varies between 6.74 and 7.36 during the follicular cycle in non-pregnant camels but it becomes more alkaline during early pregnancy, increasing from pH 7.05 after mating to as high as 8.2 at the beginning of the sixth week of gestation [3].

iii) Flow Elasticity and Vaginal Smears - Flow elasticity values and vaginal smear patterns and temperatures show no differences between pregnant and non-pregnant animals [3].

iv) Specific Gravity - This was measured using the copper sulphate method and was found to vary between 1.004 and 1.008 during the follicular phases in the non-pregnant animal. During pregnancy the specific gravity also increased, rising from 1.009 after mating to 1.014 at the beginning of the sixth week [3].

However none of these methods mentioned above are very practical under field conditions.

### **Rectal Palpation**

Diagnosis of pregnancy using rectal palpation can represent some risks to the females such as rectal tears but it is not considered detrimental to the foetus provided the examination is carried out by experienced personnel and the uterus is not over manipulated. The membrane slip test, described in cattle pregnancy diagnosis, is not possible in camelidae because of the diffuse type of placentation [4-7]. Therefore positive pregnancy diagnosis can only be achieved if the CL and foetus are palpated.

The earliest sign of pregnancy is the persistence of the CL which continues to grow until day 35 of pregnancy. It is usually soft, flabby and spherical in shape, measuring about 25 mm in diameter, but becomes out of reach after about 90 days [8].

It is not until about day 45 that uterine changes due to pregnancy can be detected by rectal palpation and the first sign is an increase in the diameter of the left horn. However, it is not until approximately the third month of pregnancy that the gravid horn feels obviously bigger and softer than the non-gravid horn and the uterus becomes more abdominal as the amount of foetal fluid increases. The cervix is pulled forward and lies just at the brim of the pelvis at 4 months, and by the fifth month the uterus is completely in an abdominal position with a small degree of fluctuation, but the foetus is not always palpable.

From the 6 month onwards the foetus can be palpated, first by ballotement, then the head and legs become easily palpable as the foetus starts its ascent. By the 9th month movement can be observed by inspection of the right flank of the animal and external signs such as an enlarged abdomen and udder are visible from about the 11th month. Precise estimation of the stage of pregnancy by rectal palpation in the dromedary is not possible beyond 3 months because of the absence of structures such as cotyledons and difficulty in reaching the foetus in this species [8,9].

Pregnancy diagnosis in the Bactrian camel by rectal palpation is similar to that of the dromedary. The first sign indicating pregnancy is a persistent corpus luteum, but it is not until 45 days that the first palpable changes in the uterus are noticed. At around this time the left horn of the uterus increases in size and is almost continuous with the uterine body. Between 2.5 - 3 months the tip of the left horn is out of reach but the bifurcation between the uterine horns can still be felt, but this is also out of reach by 4 months. By 5 months the uterus is in an abdominal position and the foetus can only be balloted in a small proportion of females. A fremitus can also be detected on the left uterine artery in 75% of the females. By 8 months the foetus becomes more easily palpable and the artery fremitus is felt on both sides. Foetal activity seems to increase from the 9th month and from 11th months to term the foetus is high and always palpable [10].

### Ultrasonography

Realtime ultrasonography, using a 3.5 or 5 MHz transrectal linear array transducer is now regarded as the method of choice for detecting pregnancy and monitoring early foetal development in large domestic animal species.

In camelids, pregnancy diagnosis by ultrasonography is possible as early as 17 days of gestation. This diagnosis is based on two main criteria, the visualization of an embryonic vesicle and presence of a corpus luteum. The corpus luteum has to be present to confirm pregnancy status unless the female is getting exogenous progesterone. In the early stages of pregnancy the embryonic vesicle is relatively difficult to visualize because it is elongated, the embryonic fluid is dispersed and the uterus is relaxed. The vesicle is however, almost always in the left horn and is best visualized at the tip of the horn where it is likely to have accumulated most fluid [9,11-13].

By day 17 of gestation the embryonic vesicle appears as a star-shaped small accumulation of fluid within the uterine lumen (Fig. 1a). As the stage of the pregnancy increases, the embryonic vesicle increases in size and becomes more visible and elongated in longitudinal view of the uterus (Fig. 1b) or more round in cross section (Fig. 1c). The embryo then becomes visible between days 20 - 22 as a small, echogenic speck within the fluid fixed at one pole of the vesicle (Fig. 1c) and the heartbeat becomes discernible between days 23 - 25 as a small fluttering within the echogenic speck of the foetus.



Figure 1a. Ultrasonographic images of the left uterine horn of pregnant camels. Day 17 of gestation shows the commencing accumulation of non-echoic conceptus fluids (arrowed) in the uterine lumen. - To view this image in full size go to the IVIS website at [www.ivis.org](http://www.ivis.org) . -



Figure 1b. Ultrasonographic images of the left uterine horn of pregnant camels. Day 20 of gestation, note the elongated non-echoic conceptus fluids. - To view this image in full size go to the IVIS website at [www.ivis.org](http://www.ivis.org) . -



Figure 1c. Ultrasonographic images of the left uterine horn of pregnant camels. Day 20 of gestation, note the conceptus fluids are more rounded when viewed in cross-section and the echogenic foetus just becomes visible lying on the uterine floor. - To view this image in full size go to the IVIS website at [www.ivis.org](http://www.ivis.org) . -

Between days 30 and 40, the overall diameter of the conceptus increases more rapidly due to accumulation of foetal fluids and during this period the allantochorion elongates further to occupy the remainder of the right uterine horn (Fig. 1d, Fig. 1e). Also at this time, a clear division can occasionally be seen between the amniotic fluid surrounding the foetus and the much larger volume of allantoic fluid external to this; the former tending to contain echogenic cell debris, whereas the allantoic fluid is much cleaner and non-echoic.



Figure 1d. Ultrasonographic images of the left uterine horn of pregnant camels. Day 35 of gestation. - To view this image in full size go to the IVIS website at [www.ivis.org](http://www.ivis.org) . -



Figure 1e. Ultrasonographic images of the left uterine horn of pregnant camels. Day 40, note the size of the foetus and volume of foetal fluids have increased considerably between days 30 and 40 and the yolk stalk is seen emerging from the foetus. - To view this image in full size go to the IVIS website at [www.ivis.org](http://www.ivis.org) . -

By day 55, the head, neck, abdomen and individual limb buds of the foetus can be easily identified (Fig. 1f), but beyond day 60 the foetal fluids have increased to such an extent that the foetus can no longer be viewed easily as it generally lies beyond the penetration range of a 5MHz transrectal probe.



Figure 1f. Ultrasonographic images of the left uterine horn of pregnant camels. Day 55 of gestation, the head, neck, abdomen and limb buds of the foetus are much more easily recognizable at this stage. - To view this image in full size go to the IVIS website at [www.ivis.org](http://www.ivis.org) . -

Visualization of the corpus luteum of pregnancy - The corpus luteum is the major source of progesterone during pregnancy in camels and its presence is required throughout the whole gestation period. The CL during pregnancy tends to be larger than the "cyclical" CL and can have various forms. For example it can be a large compact CL with a central echonegative core (Fig. 2a), or a large, dense, homogenous CL (Fig. 2b) or a large cavity CL (Fig. 2c). The latter are larger than the other two forms and change with advancing pregnancy to become completely echogenous with advancing luteinization.

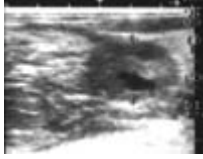


Figure 2a. Ultrasonograms of pregnant corpus lutea. Compact corpus luteum with central echonegative center. - To view this image in full size go to the IVIS website at [www.ivis.org](http://www.ivis.org) . -



Figure 2b. Ultrasonograms of pregnant corpus lutea. Dense, homogenous corpus luteum. - To view this image in full size go to the IVIS website at [www.ivis.org](http://www.ivis.org) . -



Figure 2c. Ultrasonograms of pregnant corpus lutea. Cavitory corpus luteum with a circle of echodense lutein tissue surrounding the persisting central echonegative core. - To view this image in full size go to the IVIS website at [www.ivis.org](http://www.ivis.org) . -

### Analysis Of Serum Progesterone Concentrations

Studies on progesterone concentrations during pregnancy in the camelidae confirm that these species depend on ovarian progesterone throughout their pregnancy. Ablation of the CL-bearing ovary or administration of PGF2 $\alpha$  or its analogue causes abortion or premature parturition at all stages of pregnancy, thus it would seem likely that the placenta either fails to secrete progesterone at all, or it does so in amounts insufficient to maintain pregnancy without help from the ovaries [14].

In the mated dromedary, serum progesterone concentrations increase from day 3 after ovulation to concentrations of around 3.4 ng/ml by day 8. If the camel is not pregnant concentrations rapidly return to basal levels of <1ng/ml by days 10 - 12, however, if she is pregnant the progesterone concentrations are maintained between 3 and 5 ng/ml for the first 90-100 days of gestation. According to some studies, progesterone levels then decrease slightly to 2 - 4 ng/ml where they remained until day 300. A further slight decrease then occurs over the next 70 - 80 days followed by a rapid drop to values of <1 ng/ml on the day before, or the day of parturition (Fig. 3) [15]. Other studies have shown there to be a gradual decrease in progesterone concentration from 5 months of gestation until parturition [16].

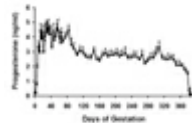


Figure 3. Mean (+sem) serum progesterone concentrations in pregnant camels. - To view this image in full size go to the IVIS website at [www.ivis.org](http://www.ivis.org) . -

The measurement of progesterone concentration in peripheral blood can thus be invaluable in the early detection of pregnancy. If a blood sample is taken between days 12 - 15 and the value is still high (i.e. >1.0 ng/ml) this would indicate that the camel is possibly pregnant. If the value has dropped to <1.0 ng/ml then the camel is definitely not pregnant.

### Other Hormone Concentrations Throughout Pregnancy

**Oestradiol-17 $\beta$**  - Serum oestradiol-17 $\beta$  concentrations show a first definite increase around day 20 - 25 after ovulation and continue to rise until concentrations of around 100pg/ml are reached between days 60 - 70 (Fig. 4) [15,17]. This increase in rate of secretion of oestrogen could be ovarian or placental in origin. An ovarian origin could be justified by the fact that follicular activity in the dromedary is not inhibited until 6 months of pregnancy [18,19] although mature follicles (<10 mm) are not generally found beyond 105 days of pregnancy [18,19]. However, a placental origin seems more likely as it has been shown that extra embryonic membranes of the camel conceptus possess considerable aromatizing capacity from as early as day 10 after ovulation and the endometrium also has the ability to conjugate the free oestrogen [20]. The timing of the oestrogen increase in the final 70 - 80 days in the pregnant dromedary coincides with the important period of increase in foetal weight and foetal fluid volume between 9 and 12.5 months [18] giving rise to the possibility of placental

oestrogens being important for fetal growth.

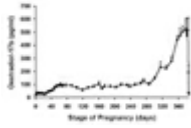


Figure 4. Mean ( $\pm$ sem) serum oestradiol-17 $\beta$  concentrations in pregnant camels. - To view this image in full size go to the IVIS website at [www.ivis.org](http://www.ivis.org) . -

**Oestrone sulphate** - In dromedaries the oestrogen sulphate concentrations show two definite peaks of about 10ng/ml in early gestation. The first peak occurs around day 26 and the second around day 70 (Fig. 5) [15]. The marked increase in oestrogen levels in the maternal blood observed after day 300 of pregnancy in the dromedary is accompanied by a decrease in progesterone and leads to a change in the ratio of oestrogen to progesterone in favour of the former, which is probably necessary for the preparation of the uterus for a normal parturition. Without this change, normal softening of the cervix and the ability of the uterus to contract during parturition are severely impaired.

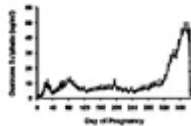


Figure 5. Mean ( $\pm$ sem) serum oestrone-sulphate concentrations in pregnant camels. - To view this image in full size go to the IVIS website at [www.ivis.org](http://www.ivis.org) . -

**13,14 dihydro-15-keto prostaglandin F2 $\alpha$  (PGFM)** - Secretion of prostaglandin F2 $\alpha$  in the dromedary, as revealed by measurement of its metabolite PGFM, remains between approximately 100 - 200 pg/ml during the first 320 days of gestation, thereafter over the next 50 days it rises sharply to around 1000 pg/ml, before a further explosive increase to peak values of 2000 pg/ml on the day of calving [15].

**Thyroid hormones** - Thyroid hormones play an important role in modulating metabolic activity, growth and differentiation of vital organs. The average peripheral concentrations of T4 (thyroxine) and T3 (Triiodothyronine) in pregnant dromedaries varies from 76 to 116 ng/ml and from 0.7 to 1.3 ng/ml respectively [21].

**Relaxin** - Relaxin is probably secreted by the feto-placental unit and is implicated in the growth of the uterus during pregnancy and relaxation of the ligaments and cervix at the end of pregnancy.

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