

قسم بحوث الكيمياء الحيوية
معهد بحوث صحة الحيوان - الدقي
مديرا القسم : أ.د / محمود عادل سليمان

التغيرات الحيوكيميائية المصاحبة للاجهاض الفطري في الماعز

بديرا عجاج ، رؤوف حلمي ، فريال الفارا ، محمد عيد
نادية محمد ، أحمد مصطفى

تم عزل كل من فطر الفيوزيريوم جرامينيوم ، كلادوسبوروم والبنسيليوم من عليقة
مركزة استخدمت في تغذية الماعز أثناء فترة الحمل *

وقد أدى ذلك الى اجهاض عدد ١٦ ماعز من ١٣٢ رأس وقد أوضحت التحاليل
الحيوكيميائية لعينات المصل المأخوذة من الماعز التي أجهضت بالمقارنة بمشلتها
التي ولدت ولادة طبيعية (زيادة في كل من البروتين الكلي والجلوبيولين وكذلك نقص
في نسبة الالبومين / جلوبيولين وكذلك نقص في الالفا ثنائي بروتاجلاندين ، بينما
لم تظهر تغيرات جوهرية لكل من الكالسيوم والفسفور الغير عضوي والكاروتين وفيتامين
(أ) وفيتامين (هـ) والبروجوستيرون والاستراديول ١٧ ب بين المجموعتين) *

Dept. of Biochemistry,
Animal Health Research Institute, Dokki,
Head of Dept. Prof. Dr. M.A. Soliman.

**SERUM BIOCHEMICAL CHANGES IN RELATION TO MYCOTIC
ABORTION IN GOATS**
(With One Table)

By
B.I. AGAG; R.H. YOUSSEF; F. EL-FAR; M.A. EID;
NADIA M. ALY and A.S. MOSTAFA
(Received at 2/9/1987)

SUMMARY

Fusarium graminearum, *cladosporium* spp. *penicillium* spp. were isolated from concentrated ration fed to pregnant goats. Sixteen goats out of one hundred - thirty two aborted. Biochemical analysis of sera from aborted and normal parturient goats, revealed a significant increase in total protein and total globulins and a significant decrease in albumin/globulin ratio and prostaglandin F_{2a} in the first group than the other one.

Serum calcium, inorganic phosphorus, Carotein, Vitamin A, Vitamin E, progesterone and estradiol 17-B showed no significant variance between the two groups.

INTRODUCTION

Abortion in goats as in other large domestic animals is due to a wide variety of infectious and non-infectious agents. In most flocks an incidence of one to five percent abortion is considered acceptable (WATSON, 1962). Higher rates of abortion should be carefully investigated.

Fungi have been rarely described as a cause of abortion in goats and ewes. In Japan, pregnant goats aborted after feeding wheat infected with *Fusarium graminearum* (URAGUCHI, 1971). CYSEWSKI, PIER and RICHARD (1968) detected mycotic abortion in ewes produced by *Aspergillus fumigatus*. In cattle, the incidence of mycotic abortion varied from 0.5 to 16 percent of all abortions.

Hence, the disease was reported in many parts of the world and many species of *Aspergilli*, yeast and other pathogenic fungi were incriminated as the causation of bovine abortion (AINSWORTH and AUSTWICK, 1973). Other reports (WILLIAMS; SHREEVE; HEBERT and SWIRE, 1977; LAING, 1979 and SINHA; SHARMA and MEHROTRA, 1980) indicated that *Aspergillus* species were most commonly associated with mycotic abortion followed by *Mucor* and *Absidia*.

Regarding the blood biochemistry and abortion in goats, MORGENTHAL (1966) studied the haematology of the Angora goat with special reference to habitual abortion. ZAGHLOUL, ABDEL-AAL and NAFIE (1985) investigated the blood biochemical alterations of goats with chlamydial abortion. On the other hand, no systemic study in relation to mycotic abortion was performed. In this investigation we recorded the first attempt to isolate fungi as a causative agent of abortion in goats and correlate this finding with serum biochemical changes.

B.I. AGAG, et al.**MATERIAL and METHODS**

Sixteen goats out of a one hundred - thirty two pregnant does in the Breeding Station at Sakha, kafr El-Sheikh province, aborted (12.12%), three to four months of gestation, during the period of December 1984 to January 1985. They were of various parities and breeds (Demashky, Albine and Balady).

High incidence of abortions occurred in Demashky breed (9 out of 38, i.e. 56.25%). While the incidence of abortions in the other two breeds, Albine and Balady, were three out of total 56 (5.36%) and four out of total 38 (10.53%), respectively. The animals are of good healthy condition without any symptoms of illness except congestion of the vulva in some aborted cases. Retention of placentas occurred only in one aborted case. The animals were kept in open sheds away from kids and fed concentrates and Trifolium alexandrium.

Swabs from vaginal discharge and parts of placenta were obtained for microbiological and viral examinations. Samples from concentrates were tested for fungi. Serum samples were taken from all the aborted goats plus five parturient goats for the microbiological (brucellosis), viral (Rift Valley fever) and biochemical investigation. These samples were obtained within 24 hours after abortion or parturition. Blood smears were also taken for parasitic examination.

procedures used in this investigation were:

<u>Parameter</u>	<u>Reference</u>
Total protein, albumin and globulin	Weichselbaum (1946) and Bartholomew and Delaney (1966).
Calcium	Glindler and King (1972).
Inorganic phosphorus	Kilchling and Freiburg (1951).
Carotein and Vitamin A	Kaser and Steko (1943).
Vitamin E	Quaife and Biehler (1945).
Progesterone	Sharma (1972).
Estradiol 17-B	Buster and Abraham (1975).
Prostaglandin F _{2a} (PGF _{2a})	Cohn, Johansson, Wide and Gemzell (1970).
Mycological isolation	Halely and Callaway (1979).
<i>Fusarium graminearum</i> identificatin	Wyllie and Morehouse (1978).
Statistical analysis	Snedecor and Cochran (1967).

RESULTS

No specific microorganisms or virus were isolated from the taken samples.

Blood smears showed no parasitic infestation. Fungi were isolated in pure culture from the concentrated ration. These included *Fusarium graminearum*, *cladosporum* spp. and *penicillium* spp.

Serum biochemical analysis (table 1) revealed a significant increase in the total protein and total globuline and decrease in albumin/globulin ratio in aborted goats than in normal parturient ones.

Hormonal values in aborted goats were the same as in normal birthes except PGF_{2a} which was not increased and the difference was significant ($P < 0.01$). No significant variance was recorded in serum calcium, inorganic phosphorus, carotein, vitamin A and vitamin E, between aborted and normal parturient goats.

MYCOTIC ABORTION IN GOATS

DISCUSSION

Fungi isolated from concentrates (*Fusarium graminearum*, *cladosporium* spp. and *penicillium* spp.) were strongly implicated as the causative agent of abortion in goats. This finding was in accordance with that reported in goats (URAGUCHI, 1971), in ewes (CYSEWSKI, *et al.* 1968) and in cows (WILLIAMS, *et al.* 1977; LAING, 1979 and SINHA, *et al.* 1980). LIW, XIE, WANG, LI, LIU, LIU, CAO and ZHANG (1985) reproduced clinical symptoms and pathological changes in sheep and goats with *Fusarium*, *Aspergillus* and *Penicillium* from forage. Herein, the isolates exercise their pathogenic effect by invasion of the foetal and placental tissues and interference with the nutrition of foetus and foetal death has been occurred. It is also possible for abortion to be the result of mycotoxicosis. CHRISTENSEN, NELSON and MIROCHA (1965) and MIROCHA, CHRISTENSEN, NELSON, SIMONELLA and STANZANI (1967) determined that *Fusarium graminearum* produced an oestrogenic metabolite like substance. MARCATO, ACCIARRI, SIMONELLA and STANZANI (1972) induced severe uterine hypertrophy in immature female mice by injection of pure culture of *Fusarium*, *Aspergillus niger* and *penicillium*. KALLELA and ETTALA (1984) reported that early abortion in cows caused by the oestrogenic *fusarium* toxin (Zearalenone) in hay.

Exogenous oestrogen represented by the mycotoxin of *fusarium graminearum* was responsible for the increase of plasma estradiol 17-B in our aborted cases. Its level was similar to that observed in spontaneous labour (table 1), which led to luteal regression and withdrawal of the plasma progesterone.

The source of PGF_{2a} appearing before foetal delivery is not known but a placental origin seems likely. It may be released in response to an increased influence of oestrogen (CURRIE, COX and THORBURN, 1976) or perhaps less likely directly in response to chronic exposure to increased corticosteroids. The significant low level of PGF_{2a} in aborted cases in our study (10.50±1.528 ng/ml) may be due to immaturation of adrenal cortex of foetal goats in this stage of gestation (CURRIE and THORBURN, 1977).

The plasma progesterone concentration determined in this study (table 2) for post-parturient goats was about 0.53 ng/ml which is similar to values reported previously (UMO, FITZPATRICK and WARD, 1976 and CURRIE and THORBURN, 1977). The aborted goats had a progesterone concentration that was indistinguishable from that of the goats delivered normally. Apparently, the decrease in progesterone concentration acts as a trigger for the start of uterine contractions which are necessary for expulsion of the foetus. The withdrawal of progesterone which precedes the foetal delivery is clearly indicative, in the goats of luteal regression (CURRIE and THORBURN, 1977). The events which are clearly in common are the major increase in plasma corticosteroids in the foetus and the episodic appearance of PGF_{2a} in the utero-ovarian vein ipsilateral to the ovary with the corpus luteum. The level of the plasma PGF_{2a} in this study was 30.84±5.55 ng/ml in normal birth. CURRIE and THORBURN (1973) demonstrated that the concentration of PGF in utero-ovarian plasma at the time of luteal regression is 5-25 ng/ml providing a potent luteolytic signal in goats.

The significant increase in serum total protein and its globulin fraction (table 1), indicated the presence of infectious agent. The effect on vitamin A level was only obvious in Demashky breed which significantly decreased than in normal birthes, 72.93±4.58 VS. 106.63±1.39 IU/100 ml. Lindburg, GROHN and KARPPANEN (1985) suggested that changes in vitamin A metabolism are caused by *fusarium* mycotoxins in feed.

REFERENCES

- Ainsworth, G.C. and Austwick, P.K.C. (1973): Fungal diseases of animals. Commonwealth Agric. Bureaux, Faraham Royal, Slough England.
- Bartholomew, R.J. and Delaney, A.M. (1966): Proceedings of the Australian Association of Clinical Biochemists, 1, 214. Cited by Wotton, I.D.P. and Freeman, H. (1982): Microanalysis in Medical Biochemistry. 6th Ed. Churchill Livingstone, Edinburgh, London Melbourne and New York.
- Buster, J.E. and Abraham, G.E. (1975): The application of steroid hormone (RIA) to clinical obstetrics. *Obstet. Gynec.* 46, 849.
- Christensen, C.M.; Nelson, G.H. and Mirocha, C.J. (1965): Effect on the white rat uterus of a toxic substance isolated from *Fusarium*. *Appl. Microb.*, 13, 653-659.
- Cohn, L.U.; Johansson, E.D.B.; Wide, L. and Gemzell, C. (1970): *Acta Endocr.* (Copenhagen), 63, 246.
- Currie, W.B. and Thorburn, G.D. (1973): Induction of premature parturition in goats by prostaglandin F_2 administered into the uterine vein. *Prostaglandins* 4, 201-214.
- Currie, W.B. and Thorburn, G.D. (1977): Parturition in goats: studies on the interactions between the foetus, placenta, prostaglandin F and progesterone before parturition, at term or at parturition induced prematurely by corticotrophin infusion of the foetus. *J. Endocr.* 73, 263-278.
- Currie, W.B.; Cox, R.I. and Thorburn, G.D. (1976): Release of prostaglandin F, regression of corpora lutea and induction of premature parturition in goats treated with estradiol 17-B. *Prostaglandins*, 12, 1093-1103.
- Cysewski, S.J.; Pier, A.C. and Richard, J.L. (1968): Mycotic abortion in ewes produced by *Aspergillus fumigatus*. *Am. J. Vet. Res.*, 29, 1135.
- Glindler, E.M. and King, J.D. (1972): Rapid colorimetric determination of calcium in biological fluids with methylene blue. *AM. J. Clin. Path.*, 58, 336-342.
- Halely, L.D. and Callaway, C.S. (1979): *Laboratory Methods in Medical Microbiology*. Published by center for Disease Control. New Publication No. (CDC) 79-8361.
- Kallela, K. and Ettala, E. (1984): The oestrogenic fusarium toxin (Zearalenone) in hay as a cause of early abortions in the cow. *Nordisk Veterinary Medicine*, 36, 305-309.
- Kaser, M. and Steko, J.A. (1943): *J. Lab. Clin. Med.*, 28, 904. Cited by Varley, H.; Gowenlock, A.H. and Bell, M. (1976): *Practical Clinical Biochemistry*. 5th Ed., 2. Williams Heinemann Medical Books Ltd., London.
- Kilchling, H. and Freiburg, Br. (1951): Inorganic phosphorus in serum. In *Clin. Photometria*, 3rd Ed. Wiss Verl. Ges. mbH, Stuttgart.
- Laing, J.A. (1979): *Fertility and Infertility in Domestic Animals*. 3rd Ed., pp. 239-241. The English Language Book Society and Bailliere Tindall.
- Lindburg, L.A.; Grohn, Y. and Karppanen, E. (1985): Massive lipid accumulation in mink liver stellate cells may be caused by *Fusarium* mycotoxins in the feed. *Acta Vet. Scand.*, 26, 423-424.
- Liu, X.Y.; Xie, Y.F.; Wang, Z.X.; Li, C.S.; Liu, X.H.; Liu, S.L.; Cao, Y.X. and Zhang, W.Z. (1985): Causes of black, watery diarrhoea among cattle in Taibai country, Shaanxi Province, China *Chinese J. Vet. Sci. and Techn.* 4, 20-23.
- Marcato, P.S.; Acciari, C.; Simone, P.; Simone, A. and Stanzani, F. (1972): Oestrogenic activity in female mice of strains of *Aspergillus niger*, *Fusarium* and *Penicillium* isolated from mouldy maize which had been responsible for hyperoestrogenism in sows and rabbit does. *Nuova Veterinaria*, 48, 225-264.
- Mirocha, C.J.; Christensen, C.M. and Nelson, G.H. (1967): Estrogenic metabolite produced by *Fusarium graminearum* in stored corn. *Appl. Microb.*, 15, 497-503.

MYCOTIC ABORTION IN GOATS

- Morgenthal, J.C. (1966): The haematology of the Angora goat with special reference to the habitual aborter. I. The pregnant doe. Onderstepoort J. Vet. Res., 33 (1), 363-378.
- Quaife and Biehler (1945): J. Biol. Chem., 159, 633. Cited by Oser, B.L. (1979): Hawk's Physiological Chemistry. 14th Ed., Tata McGraw-Hill Publishing Company Ltd., New Delhi.
- Sharma, S.C. (1972): A sensitive RIA progesterone F. J. Physiol. 226, 74.
- Sihna, B.K.; Sharma, T.S. and Mehrotra, V.K. (1980): Fungi isolated from the genital tract of infertile cows and buffaloes in India. Vet. Rec., 106, 177-178.
- Snedecor, G.W. and Cochran, W.G. (1967): Statistical Methods. 6th Ed. Iowa State University Press, Ames.
- Umo, I.; Fitzpatrick, R.J. and Ward, W.P. (1976): Parturition in goats: Plasma concentrations of prostaglandin F and steroid hormones and uterine activity during late pregnancy and parturition. J. Endocr., 68, 383-389.
- Uraguchi, K. (1971): Mycotoxins of Fuzarian. In Internal Encycl. of Pharmacology and Therapeutic (M. Raskova ed.), 2, Sec. 71, Pergamon Press, New York.
- Watson, W.A. (1962): Ovine abortion. Vet. Rec., 74, 1403.
- Weichselbaum, T.E. (1946): An accurate and rapid method for determination of proteins in small amounts of blood serum and plasma. Am. Chem. Path., 10, 40-45.
- Williams, B.M.; Shreeve, B.J.; Hebert, C.N. and Swire, P.W. (1977): Bovine mycotic abortions: Some epidemiological aspects. Vet. Rec., 100, 382.
- Wyllie, T.D. and Morehouse, L.G. (1978): Mycotoxic Fungi, Mycotoxins, Mycotoxicosis. An encyclopedic handbook. Published by Marcel Dekker, Inc. New York and Basel.
- Zaghloul, A.H.; Abdel-Aal, Th.S. and Nafie, Th.S. (1985): Some biochemical alterations in the blood serum of goats with chlamydial abortion. In Abstracts of the Second National Congress of biochemistry, Cairo, pp. 123-124.

Table (1)
Serum biochemical parameters in normal parturient and aborted goats

Parameter	Normal parturient goats	Aborted goats
Progesterone (ng/ml)	0.530 \pm 0.022	0.576 \pm 0.017
Estradiol 17-B (pg/ml)	249.400 \pm 1.280	246.440 \pm 1.340
Prostaglandin F _{2a} (ng/ml)	30.840 \pm 5.550	10.500 \pm 1.530**
Total protein (gm/100 ml)	7.390 \pm 0.840	8.590 \pm 0.290*
Albumin (gm/100 ml)	2.730 \pm 0.110	2.660 \pm 0.089
Globulin (gm/100 ml)	4.660 \pm 0.330	5.860 \pm 0.240**
A/G Ratio	0.590 \pm 0.027	0.460 \pm 0.021**
Calcium (mg/100 ml)	10.340 \pm 0.370	10.140 \pm 0.290
Inorganic-P (mg/100 ml)	6.890 \pm 0.460	6.800 \pm 0.360
Carotenoids (ug/100 ml)	11.730 \pm 0.770	12.540 \pm 0.590
Vitamin A (I.U./100 ml)	106.630 \pm 1.890	100.550 \pm 7.970
Vitamin E (ug/100 ml)	715.000 \pm 27.500	726.770 \pm 21.890

Mean \pm Standard error.

* : Significant at P/ 0.05.

** : Significant at P/ 0.01.