Immuno- Genetic Pathological Study of Protozoal Abortion in Aborted Fetus's and Placenta of Iraqi Cattle.

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Abstract:

Current study was conducting on 50 tissues samples which were on 25 placentas from aborted cow and25 from aborted fetuses. Each tissues sample was cutting in to 4 pieces about 1cm; 1st one kept in 10% formaldehyde and sent to pathology laboratory and 2nd kept in clear test tube and sent to microbiology laboratory in Veterinary Medicine College of Baghdad University. Third and four were sent to laboratory of molecular and immunohistochemistry(IHC) to detection DNA defects; P^{53} (apoptosis factor) and TNF (tumor necrotic factor) a markers on placental and fetal tissues. Further to serological test for hormones in all aborted female. Microbial examination showed positive test for bacterial and protozoa causes. Conclusions pathological findings in aborted fetus mainly were: in brain; neuronal demyelination and in liver; hepatocytes' vacoulation and inflammation and in lung: edematous lesion with inflammation; and in placenta necrotic foci with abscesses. Genetics test showed DNA damage in brain; liver of fetus and placenta. Immunological examination after IHC methods showed that there was detectable increasing in ratio of TNF and P^{53} in placental and fetal tissues. Serological test showed increase serum level of estrogen hormone and a prolactin hormone.

Key Words: Protozoa, abortion, cattle, Iraq.

دراسة مناعية وراثية مرضية في أجهاض الاوالي في المشيمة والاجنة المجهضة في الابقار العراقية

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المستخلص

تضمنت الدراسة الحالية على 50 عينات نسيجية منها 25مشيمة معها 25الاجنة المجهضة في الابقار العراقية.كل عينة نسيجية قسمت الى 4 اجزاء حوالي 1سم ، الاولى حفظت في 10%فورمالديهايد وارسلت الى مختبرالامراض ، الثانية حفظت في انبوب مختبري صافي وارسلت الى مختبرالمايكروبات.الثالثة والرابعة ارسلت

الى مختبرالجزيئي والمناعي النسيجي للكشف عن عيوب الدنا وعنP^{53} المؤشر الخاص بحصول موت الخلية المبرمج ، وعن المؤشر TNFالخاص بحصول التنخر في انسجة المشيمة والجنين .اضافة الى فحوصات السيريولوجية الهرمونية في كلية الطب البيطري جامعة بغداد .التشخيص المختبري المايكروبي أظهروجود اصابة بكتيرية مع اصابة طفيليات الشبيهة بالاوالي .الاستنتاج وجودعلامات مرضية في الاجنة المجهضة اهمها في الدماغ نقص النخاعيين والخلايا العصبية وفي الكبد تفجي الخلايا الكبدية مع التهاب وفي الرئة وذمة مع التهاب مع بؤر نخرية وتقييح في المشيمة الفحوصات الوراثية أظهرت تلف الدنا في الدماغ والكبد والمشيمة . التشخيص المناعي بطريقة المناعة النسيجية أظهرت زيادة ملحوضة في مؤشرات النخر والموت المبرمج (P^{53};TNF)في انسجة الاجنة والمشيمة .الفحوصات السيريولوجية أظهرت زيادة مستوى المصل الدموي لهرمونات الاستروجين والبرولاكتين.

الكلمات المفتاحية: الاوالي، الاجهاض ، الابقار ، العراق .

Introduction

Abortion is the premature expulsion of the fetus from the dam and usually occurs because the fetus has died in uterus. If death occurs at 1-2 months of gestation, it is usually termed “early embryonic death.” This embryo or early stage fetus is usually just resorbed by the uterus with no signs. After 2 months of gestation, there is usually the expulsion of the fetus and placental tissues. Some agents which can cause abortion may instead result in the birth of a live but weak newborn, or with congenital defects (anatomical or physiological defects present at birth) (5). Embryonic and fetal deaths deprive the fetomaternal unit of whatever contribution the conceptus makes to the continuation of pregnancy.(5) Parturition and presumably abortion in most animal species is initiated by the fetal adrenal gland. Signal, perhaps a stressful event causes the fetal pituitary to secrete Adrenalin(ACTH) Corticosteroid Hormone that in turn results in the glucocorticoid production by the adrenal gland corticosteroids increase the synthesis of estrogens in the placenta, which in turn causes the synthesis and release of Prostaglandins (PGF2α)from the endometrium and myometrium this causes luteolysis and a decreased progesterone production. (5) In large animal the losing of very small embryos doesn't influence the time of return to estrus. The next estrus will be somewhat increase because the corpus luteum will have been programed for prolongation of its life.(5).Regardless of the sources of hormones responsible for maintaining pregnancy in large animals, embryonic or fetal death permits the release of PGF2α and expulsion of the embryo or fetus. (5) The exact outcome is unpredictable and is influenced, among other things by species, stage of gestation, and number of fetuses .In the bitch and queen, the life span of the corpus luteum is not very different between pregnant and no pregnant animal; when embryonic or fetal death occur in domestic carnivores. The demise of the corpus luteum is definite and dead products of conception may be retained until approximately the normal time of parturition fetal autolysis is therefore common.(5) Neosporosis consider as protozoal causes for abortion in cattle the general name is Coccidiosis, *Neospora caninum* isolated from

aborted cattle and fetus. The histopathological lesions mainly were multiple necrotic foci with meningitis and encephalomyelitis in brain and co emulative necrosis appear as necrotic foci in liver in fetus; with necrotic foci on placenta.(14) Spore of *Neospora caninum* infected brain and different organs of fetus and offered congenital risk due to damaging of DNA which interfering with normal development of fetus.(14).Placental necrosis accompanied by heavy inflammatory reaction resulted in hormonal misbalances that end with abortion.(13). Aims of this study are immunopatho-genetics examination of abortion cause in Iraqi cattle specially report abortion due to infection with *Neospora caninum* ; and report DNA damage in Placenta ; and report presence of spores of *Neospora caninum* in within aborted fetus.

Material and methods: **1**-Tissues samples: 50 tissues samples conducted on 25 placenta from aborted cow and 25from aborted fetuses' each one were divided to 4 pieces about 1cm; 1st one kept in 10% formaldehyde and sent to pathology laboratory and 2nd kept in clear test tube and sent to microbiology laboratory in. 3rd one kept in 10% normal saline diluent formaldehyde and sent to laboratory of molecular for detection DNA defects uses FISH test.4th one kept in10% formaldehyde and sent to immunohistochemistry laboratory for estimation P^{53} and TNF a markers on placental and fetal tissues**.2-**Blood Sample: Samples of blood were collected from 25 aborted cattle and were sent to laboratory for serological tested for detection of estrogen and prolactin hormones value.

Results and Discussion: **1-**Immunohistopathological Results: Application of Immunohistochemistry technique on tissue samples according to author (1). Results showed increase percent of P^{35} and TNF in placenta and fetus organs (liver and brain) and placenta tissues. Figure (1).

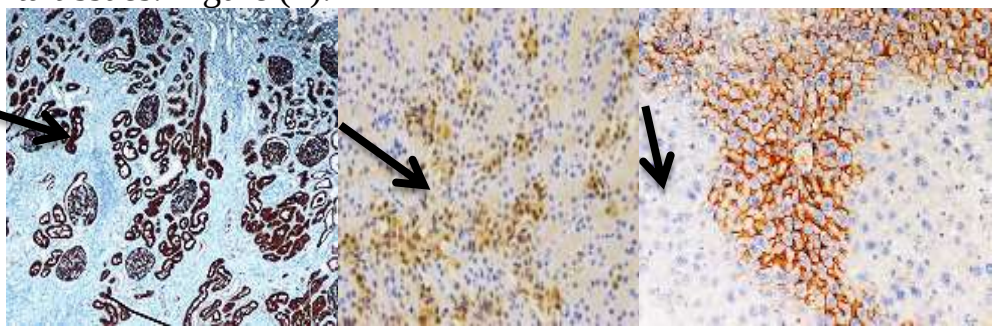

Figure (1, A-B-C) A: Placenta B: Liver C: Brain.

Abortion in cattle in advances period (6-7-8) months of pregnancy occurs mainly due to virulent pathogen *Neospora* which make changes in normal apoptosis mechanism and associates with control necrosis marker besides its effects on genetic material(13) (3) .

2-Results of microbial examination: report infected placenta and lung and liver of fetus with pathogen *Neospora caninum* (8)(3)(10). And make IMC staining (13).in immunity laboratory. Figure (2).

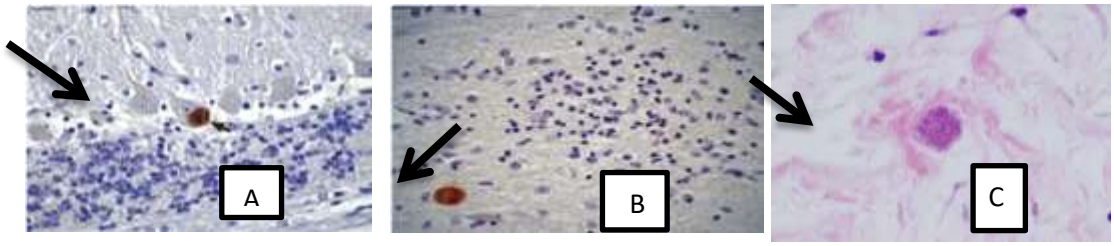

Figure (2, A-B-C) A: meninges; B: brain; C: Placenta

3-Results of histopathological changes: Grosse picture of placenta and fetus showed 8th month age aborted fetus and placenta with hemorrhagic and necrotic foci .Figure (3). Histopathological finding showed inflammatory and granulomatous reaction around spores of *Neospora caninum* with fibrous band of connective tissue in both aborted placenta and fuse's brain. Figure (4).

Figure (3A-B).Gross picture of aborted 8th age fetus and aborted placenta

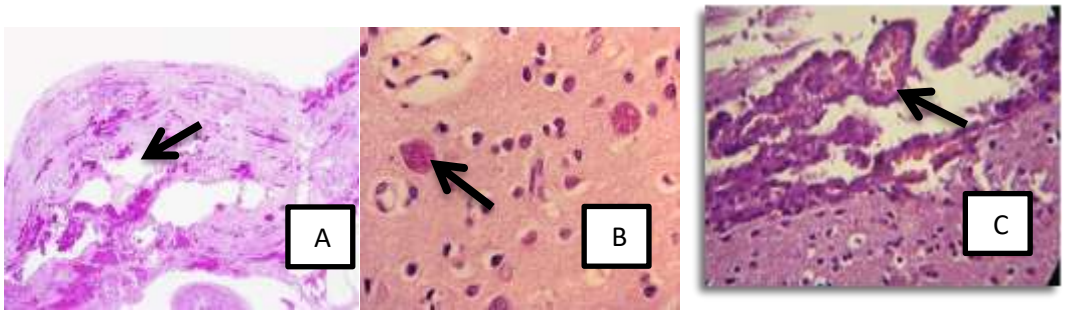

Figure (4A-B-C): A: placenta with necrotic foci; B: brain showed spore C: brain showed hemorrhagic destructive changes

Abortion occurs mainly at 8th month of pregnancy association with signs of chronic inflammation characterized evidence of giant cell (adhesive macrophages) presence with fibrous proliferation .Figure (5) .

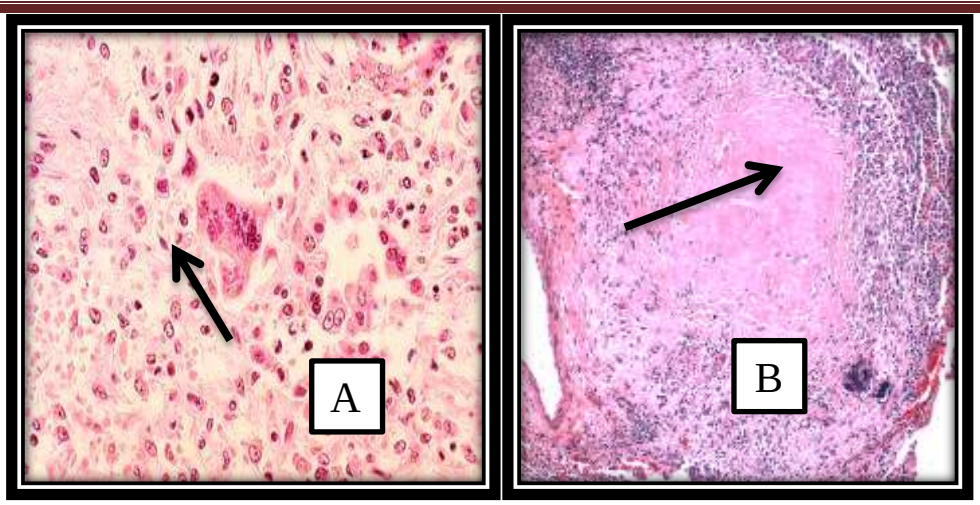

Figure (5) showed: **A**: placenta of aborted cow showed giant cell (arrow).B: granuloma (arrow)

4- Results of molecular test. Placental tissue were prepared to make Fluorescence in situ hybridization, procedure were don according to author (1).result showed infected placenta with spores of pathogen appear green fluorescence and there are high scoring percent for infection. Figure (6) .

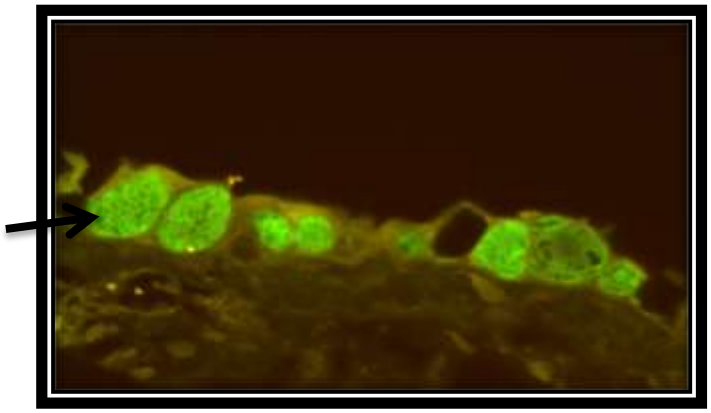

Figure (6).Placenta of aborted cow showed fluorescence green spore (arrow) of pathogen with high incidence.

5- Results of hormonal estimation: blood samples collected from aborted cow showed elevation of estrogen and prolactin level more than normal value during 8th month of gestation period which consider as marker for hormonal disturbances due to inflammation on placental which certainly effect and stimulates ovary and uterus to produced furthered hormones.

Figure (7).showed increase level of estrogen and prolactin in blood during abortion time.

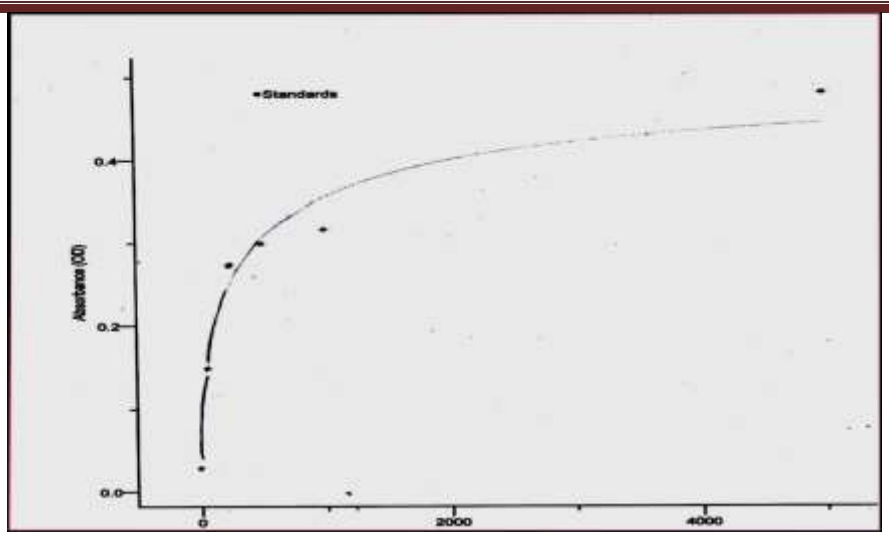

Figure (7).showed elevation in serum level of both estrogen and prolactin during abortion time (8th month).

Abortion causes in Iraq are mainly bacterial but in this study report that infected cattle with *Neospora sp*. Caused abortion in advanced stage of pregnancy at 8th month of gestation period diagnosis and isolation depend on tissues picture with special microbial test for *Neospora sp.* Identifying(3)(11).Immunological and molecular previous researches were used for detection of infection with *Neospora* sp.(11)(4).which improved the harmful effects of pathogen on tissue structure specially DNA damage.(13).Pathological finding on aborted placenta and fetuses tissues were granulomatous inflammation due to chronicity associated with necrosis due to virulent pathogen.(2).brain of fetus infection refer to pass placenta blood barrier indicating low immune response represented by increase P53 marker responsible for apoptosis and TNF tumor necrotic factor responsible for increase necrotizing lysosome.(7). Estrogen level elevated responsible for expulsing of fetuses and increase serum prolactin level (8)(9).Presence of Dog within cattle field and direct contact with food and water make chance increase incidences of infection with *Neospora caninum*.(12).

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