30	ARIPEX	ORIGINAL RESEARCH PAPER		Veterinary Medicine	
Indian			SES OF INFERTILITY IN CROSS DAIRY COWS OWNED MALL HOLDERS IN KHARTOUM, SUDAN	<b>KEY WORDS:</b> cause infertility dairy cattle	
Dr. MOAMER         HASSAN         University of Bahri Faculty of Veterinary         Gynecology.         ELFADIL		University of Bahri Faculty of Veterinary Medicine Gynecology.	e, Department of Surgery and		
Dr. AMBROSE SAMUEL JUBARA University of Bahr-El Ghazal Faculty of Veterinary Medicine Department Gynecology and Surgery			y Medicine Department of		
Dr.TAMADOR- ELKHANSAA ELNUOR ANGARA			Sudan University of Science and Technology Faculty of animal production		
ABSTRACT	The aim of this study was to determine the main causes of the infertility in cross dairy cows in Eastern Nile Locality (ENL). A total of 491 cows were followed up during the period (2008-2010) examined for general health and infertility problems by: case history, rectal palpation and serological test beside serum level of progesterone hormone analysis. The study reveals that (85cows) 17% of cross cows were infertile, follicular cysts 16.32% were the major cause of infertility (p<0.05) followed by metrits 11.8% On the other hand, cows sustaining inactive ovaries and brucellosis showed significant				

prevalence.

## Introduction

۷

Reproductive disorder became a major problem in dairy cattle production to the point that it is becoming increasingly apparent, that high yielding cows were unable to conceive under the optimum condition. This negative association between production and fertility has been observed in several dairy breeds (Pryce and Veerkamp, 2001). Ten% to 30% of dairy cows may be affected by infertility and reproductive disorders (Erb and Martin, 1980), and 3-6% of the cattle culled annually in developed countries for these reasons. The extent of the problem was likely similar in the tropics, although updating data were not available (FAO, 1989).

Sterility is a permanent factor preventing the production of new generations, and infertility or temporary sterility is the inability to produce viable young calves within stipulated time characteristic for each species (Hafez, 2000).

According to Grohn and Rajala-schulltz (2000) infertility causing diseases are the factors that influence fertility, while Lucy (2001) considered the age of the cow, production and nutrition. Whereas Veerkamp *et al.*, (2000) related them to the reproduction physiology of dairy cow.Arthur (1982) conceived that the causes of infertility are many and can be complex; they are related to Graafian follicle development and maturation, oestrus onset, successful coitus, ovulation, fertilization, implantation, and the development and delivery of the fetus and its membranes. According to Osmanu (1979), anything interfering with these routines, such as diseases, poor nutrition, inadequate herd management, hereditary and congenital factors, hormonal disturbances or environmental changes, makes the animal infertile, even if they were temporary.

The other causes of infertility included ovarian cyst and inactive ovaries with complete absence of estrus, early embryonic mortality with repeat breeding and prolonged gestation were responsible for 59.4% of the cases of infertility (Kodagali, 1974). Anoestrus often reflects a hormonal disturbance and accounted for 47.8% of the cases. According to Singh *et al.*, (1981) repeat breeding, where cows require three or more services to conceive, accounted for 11.5% of cases. The author also found functional infertility to be more common than infertility due to infectious diseases (76% vs 24%). Anoestrus is exacerbated by inadequate nutrition, high ambient temperature, and high parasitic burdens and may be due to an elevated level of prolactin (Vandeplassche, 1982).

Ovarian cyst Disease is a common and cause significant economic impact condition of dairy cows (Johnson and Coates, 2004).

There are several definitions used to describe follicular cysts, and the traditionally accepted definition is that they are defined as an ovulatory fluid-filled structure 2.5 cm in diameter that persists on the ovaries for more than 10 days (Archibald and Thatcher, 1992). Follicles normally ovulated at 1.7cm in diameter, so follicles remain at that diameter or larger may be considered to be a follicular cyst (Hatler et al., 2003). Vanholder *et al.*, (2006) suggested that cystic ovarian follicles (COF) should be defined as "follicles with a diameter of at least 2 cm that are present in one or both ovaries in the absence of any luteal tissue and that clearly interfere with normal ovarian cyclicity.

Brucellosis is one of the most known zoonotic diseases in the world (Ariza et al., 2007) and important disease of livestock (Nasir et al., 2005) which still Remains as a widely prevalent zoonotic disease of public health and economic importance to livestock owners as well as to a nation (Schelling et al., 2003, Zinsstag *et al.*, 2007)

Puerperal metritis is a severe disease that adversely affects milk production and reproduction, and the cow can have many metabolic disorders undertake potentially life (Overton, 2003). Metritis is defined as inflammation of the muscular wall of the uterus and endometrium (Okker *et al.*, 2002). The serious cases occur during the first 10-14 days postpartum and are sometimes called toxic puerperal metritis, acute metritis postpartum or puerperal metritis (Chenault *et al.*, 2004). The incidence of toxic metritis varies from 2.2% to 37.3%. Cows affected exhibit varying degrees of depression, poor appetite, and reduced milk production and are predisposed to suffer from disorders abomasums (Radostits et al., 2000 and Overton, 2003).

## Material and Methods The study Area

The study was conducted in Eastern Nile Locality (ENL), Khartoum State Republic of Sudan. The locality is located between longitude 15 37° and 15 78° North and latitude 32 32° to 32 71°East. The area represents a typical semi-arid zone with substantial variation in temperature and humidity. In dry summer months between (March-June), average monthly temperature ranges between 23.0-40.5 C°, while in wet summer (July-October) the average monthly temperature ranges between 24.5-38.7C° with average

## PARIPEX - INDIAN JOURNAL OF RESEARCH

rain fall reaching 26,08 mm. In winter (November-February) the temperature ranges between 17.0 - 33.8C°.The Humidity varies with season; it reaches an average of 23 % in winter and 22%, 45% in dry summer and autumn respectively.

The cattle in the locality are kept in farms, each of these farms had shades and fences made of local materials such as haseer, baboons iron poles were used for construction of animals' houses. Pens were divided according to the different production groups such as milking, dry pregnant cows, replacement heifers and calves. Feeds and water troughs were available in every pen. Hand milking and natural mating were used in all the farms under the study. (Some farmers use artificial insemination).

Animals were generally fed sorghum grass (Abu-70), alfalfa, sorghum straws, groundnut residues, molasses and bagas (by product of sugar industry). Concentrate given depends on the level of milk production and usually ranges between 2-6kg twice daily. Mineral blocks were available.

## Methods of data collection

Sources of data: primary data as well as secondary were used in the study.

The primary data: was collected by conducting a survey during two years (2008-2010). To collect data we needed to test the hypotheses set. The survey was conducted in small holder's dairy farms that keep crossbred cows in the study area were surveyed. Across section survey of the small holders dairy farms that keep crossbred cows was conducted. The objective of the survey was to determine prevalence and types of infertility problems among small holder farmers in ENL. Direct interview with the farmers beside the supervisor observation were used. The interview included the case history with special emphasis on breeding, clinical signs, general observations and records.

The selected farms. Twelve clusters of smallholder's dairy farms in different locations of ENL were selected depending on the consent of the farmer.

### The targeted animal population

Based on phenotypic features and direct interviews with the farmers, crossbred cows in all the selected farms were recorded. Various identification methods were used to identify the animals such as name, number and ear tag. Colours sometimes used together with descriptive feature (e.g. broken horn). The above data were either collected directly from available records or direct interview with the farmer.

# Determination of crossbred cows sustaining infertility problems

The identification of cows sustaining infertility problems was based on history, clinical signs. All animals in the selected farms were subjected to repeated clinic-gynecological examination. The diagnosis was made on the basis of history, observations, rectal palpation and serological test.

Herds under the study were visited twice a week for clinical monitoring, observations and collection of samples. All suspected animals were kept under close observations for clinical signs and other externally clear lesions. Rectal palpation as a diagnostic method was used as described by (Hafez, 2000).

## Laboratory analysis

Blood samples were collected from milk vein once a week for a period of 3weeks using plain Vaccutainers tubes. These samples were centrifuged (at 3000g for 20 min) and sera were separated. The obtained serum was divided into two parts one for diagnosis of brucellosis and the other for determination of progesterone levels after being stored under -18 C°.

For diagnosis of brucellosis, serum samples were screened using the Rose Bengal Plate Test (RBPT). The test procedure recommended by Alton et al., 1988 was followed. Briefly,  $30\mu$ L of RBPT antigen and  $30\mu$ L of the tested serum were placed alongside

on the plate, and then mixed thoroughly. The plate was shaken for 4 min and the degree of agglutination was recorded. The sample classified positive if any agglutination was observed and negative if no agglutination.

Progesterone level was assayed using ELISA kit; this was done according to manufacture instructions "Dialab produktion and und vertreb van chemisch-technischen produkten und laborinstrumenten Gesellschaft m.b.H.

A-2351 winer Neudorf, Austria, Iz-NO sud Hondastrasse, object M55."  $\,$ 

## 1- Principle:

Progesterone (antigen) in the sample competes with horseradish peroxidase-progesterone (enzyme-labeled antigen) for binding onto the limited number of anti-progesterone (antibody) sites on the micro plates (solid phase).

After incubation the bound/free separation is performed by a simple solid-phase washing. The substrate solution (H2O2 /TMB) was added. After an appropriate time has elapsed for maximum color development, the enzyme reaction was stopped and the absorbances were determined. Progesterone concentration in the sample was calculated based on series of standards. The color intensity was inversely proportional to progesterone concentration of the sample.

## 2-Proceduer:

Two wells were prepared for each of the six points of the calibration curve (CA-CE) and for each sample, one for Blank.

Samples 50µl, calibrators (CA-CE) 50µl, Conjugate 50µl for sample and calibrator were incubated at 37sC for 1 hour, the contents from each well were removed; and the wells were washed with 300µl of distilled water. The washing procedure was repeated the water was drained completely, 100µl substrate solution was add to calibrator, sample and Blank using pipette, then incubated at room temperature (20-25sC) for 15 minutes in the dark. 100µl stop solution was added to all (calibrator, sample and Blank). The absorbance was read (E) at 450nm against Blank.

The results were calculated and the concentration of samples was read.

The levels of progesterone in the collected sera were used to confirm infertility problems that resulted from hormonal imbalance. Persisting levels of progesterone > 1ng for 28 days was diagnosed as anoestrous condition. However, levels < 1ng persisting for the same period or more was considered as conditions of inactive ovaries. Fluctuation of progesterone levels indicates repeat breading or early embryonic death conditions.

## 3.3. Data Analysis

SPSS program (version 10, 2010) was used to mange and analysis the collected data. Descriptive statistics was done to explore the prevalence of infertility diseases and Pearson Chi-square test was used to determine level of significance among clinical cases of cows in different dairy farms. Chosen level of significance is p=0.05.

## **Results and discussion**

In the present study the infertility problems in cross dairy cattle accounted to (17.31%). out of which 87.05% were reported to have a known (direct) causes. whereas the cause of 12.98% of them remained unknown causes. The failure to specify the real cause of infertility of 12.98% was attributed to difficult diagnosis of these causes by using rectal palpation.

## Small holder's dairy farms survey

The survey of small holder's dairy farms involved twelve (12) farms. The total number of cows raised in these farms were 552 cows out of which 491 (88.95%) were crossbred mainly Friesian and local breeds (Butana and kenana) (Table 1).

## PARIPEX - INDIAN JOURNAL OF RESEARCH

## Volume-6 | Issue-9 | September-2017 | ISSN - 2250-1991 | IF : 5.761 | IC Value : 79.96

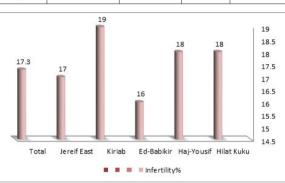
#### Table 1: Distiribution of the selected Dairy Farms (n=12)

Units	No. of farms	No. of cows	Crossbred	%
Hilat Kuku	4	153	148	97
Haj-Yousif	3	150	136	91
Ed-Babikir	3	149	135	91
Kiriab	1	40	37	93
Jereif East	1	60	35	58
Total	12	552	491	89
%	100	100	88.95	-
Mean±SD	-	46±20.82	40.92±18.86	-

The mean of crossbred cows per farm is 40.92 with a standard deviation (SD) of ±18.86 which indicates that small holder farmers in Eastern Nile Locality (ENL) prefer to raise crossbred more than indigenous cows

rable 2. Intertifity problems encountered in ENL				
location	No. of farms	Cross bred cows	Infertile cows	Infertility %
Hilat Kuku	4	148	27	18
Haj-Yousif	3	136	24	18
Ed-Babikir	3	135	22	16
Kiriab	1	37	7	19
Jereif East	1	35	5	17
Total	12	491	85	17.3

# Table 2: infertility problems encountered in FNI



## Fig. 1: infertility problems encountered in ENL

4.2 Infertility problems among crossbred cows in the selected farms.

The study revealed that 85(17.31%) cows sustained reproductive problems with a mean of 6.07±4.14 per farm (Table 3). Cows sustaining infertility problems in ENL were significantly (p<0.05) higher in Haj-Yousif (8.0 ±2.0) and Ed-Babikir (7.33 ±4.16) compared to Hilat Kuku, Kiriab and Jereif East.

High prevalence of direct reproductive problems in all farms under the study was found to be 87.05% and the indirect reproductive problems affecting reproduction was 12.98% (Table 4).

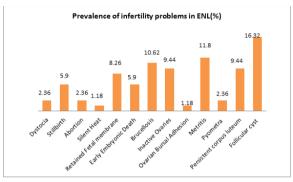
# Table 3: Types of infertility problems diagnosed in all the farms under study (n=13)

Type of Infertility	Type of Direct Infertility	Cows Affected	Prevale nce%
	Follicular cyst	14	16.32a
	Persistent corpus luteum	08	9.44b
	Pyometra	02	2.36e
	Metritis	10	11.8b
	Ovarian Bursal Adhesion	01	1.18e
	Inactive Ovaries	08	9.44b
	Brucellosis	09	10.62b
	Early Embryonic Death	05	5.90d
	Retained Fetal membrane	07	8.26c
	Silent Heat	01	1.18e
	Abortion	02	2.36e

	Stillbirth	05	5.90d
	Dystocia	02	2.36e
Direct Infertility problems		74	87.05
Indirect Infertility problems		11	12.98
Total		85	100
Mean ± SD		6.07±4.14	

a,b,c,d,e for figures within the same column bearing different superscripts differ significantly (p<0.05)

Cows sustaining follicular cysts showed significantly higher prevalence (p<0.05) compared to other infertility problems Fig.2. On the other hand, cows sustaining metritis, Inactive ovaries and brucellosis showed significant prevalence (Table 4).



#### Fig 2: Types of Infertility Problems

#### REFERENCES

- Archibald, LF. and Thatcher, W. W. (1992). Ovarian follicular dynamics and management of ovarian cysts. In: Large Dairy Herd Management. Van Horn HH, Wilcox CJ, eds. Am Dairy Sci Assoc, Champaign, IL. Ariza, J.; Cascio, A.; Colmenero, J. D.; Corbel, M. and Falagas, M. E. (2007)
- Perspectives for the treatment of brucellosis in the 21st century: the loannina recommendations. PLoS Med 4: e317 doi: 10.1371/journal.pmed.0040317.
- Arthur, G. H., (1982). Veterinary reproduction and obstetrics 5th edition Bailleire 3.
- Tindall, London, UK., 616 pp.
   Chenault, J.R.; McAllister, J.F.; Chester, S.T.; Dame, K.J.; Kausche, F.M. and Robb,
   E.J. (2004).Efficacy of ceftiofur hydrochloride sterile sus-pension administered 4 parenterally for the treatment of acute postpartum metritis in dairy cows. J Am Vet Med Assoc; 224, 1634-1639. Erb, H. W. and Martin, S.W. (1980). Interrelationship between production and
- 5 reproductive disease in Holstein cows. J. Diary. Sci., 63, 1911-1917
- 6 FAO, (1989). Infertility in cow, Areview of reproductive performance of femal Bos indicus (Zebu) cattle.fao roma italiy. www. Fao . org / wairdocs / ilri / x 5442 e/x5442e07.htm
- Grohn, Y.T. and Rajala, P.J. (2000). Epidemiology of reproductive performance in dairy cows. Animal 9. Reproduction Science. 60-61, 605 614. 7.
- 8. Hafez, E.S.E. (2000). Reproductive failure in females, Reproduction in farm animals chapter 17 page261.USA. Hatler, T. B.; Hayes, S. H.; Laranja, L. F.; De Fonseca. and Silvia, W.J.
- 9 (2003).Relationship Between Endogenous Progesterone and Follicular Dynamics in Lactating Dairy Cows with Ovarian Follicular Cysts, Biology of Reproduction., 69, 218-223
- Johnson, W.H. and Coates, A.E. (2004). An update on cystic ovarian disease. 10. Journal of Education, Science and Technology. Vol. 1 (2): pp. 65-69
- Kodagali, S.B. (1974). Report on study of Infertility in cattle 1965-69. Animal 11 Breeding Abstract., 43, 3369
- Lucy, M.C. (2001). Reproductive loss in high producing dairy cattle. J. Dairy. Sci., 12. 84. 1277-1293.
- 13. Nasir, A.A.; Perveen, Z. and Ikram-ul-Haq, M. (2005). Comparative study of standard and modified serum agglutination tests for diagnosis of Brucellosis in animals. Pakistan Vet. J., 25(1), 33-34.
- Okker, H.; Schmitt, E.J. P.and Vos, L.A.M., Scherpenisse, P., Bergwerff, A.A, Jonker, F.H., (2002).Pharmacokinetics of ceftiofur in plasma and uterine secretions and tissues after subcutaneous pos, tpartum administration in lactating dairy cows. J. Vet. Pharmacol Therap; 25, 33-38.
- 15. Osmanu, S.T. (1979). Studies on bovine infertility at the agricultural research Station (Legon) over half a decade (1972-77). Ghana University Department ofAnimal Science Studies, Legon, Ghana., 82 pp.
   Overton, M.W.; Sischo, W.M. and Reynolds, J.P.(2003).Evaluation of estradiol
- cypionate administered prophylactically to postparturient dairy cows at high risk for metritis. J Am Vet Med Assoc., 223, 846-851.
  Pryce, J.E. and Veerkamp, R.F. (2001). The incorporation of fertility indices in
- genetic improvement programmes. Pages 237-249 in fertility in the high producing Dairy Cow. M.G. Diskin, ed. British Society of Animal Science, Occasional Publication No 26. Edinburgh, Scotland.
- Radostits, O.M.; Gay, C.C.; Blood, D.C. and Hinchcliff, K.W. (2000). Veterinary Medicine. 9th ed. London: Bailliere Tindall; 322.
- Schelling, E.; Diguimbaye, C.; Daoud, S.; Nicolet, J.; Boerlin, P.; Tanner, M. and 19. Zinsstag, J. (2003). Brucellosis and Q – fever seroprevalances of nomadic pastoralists and their livestock in Chad. Prev. Vet. Med., 61, 279-293.
- Singh, C.S.P.; Singh, S.K. and Singh, B. (1981). Studies on incidence of infertility in cows. Indian Veterinary Journal., 58, 909-912 (veterinary Bulletin 51:7266). Smallholder Dairy Project Herd of Zimbabwe. Southern African 20.
- 21.Vandeplassche, M. (1982). Reproductive efficiency in cattle: A guideline for projects in developing countries. FAO Animal Production and Health Paper No.25.FAO,

## **PARIPEX - INDIAN JOURNAL OF RESEARCH**

- 22.
- Rome, Italy. 118 pp. Vanholder, T.; Opsomer, G. and Kruif, De. A. (2006).Etiology and Pathogenesis of Cystic Ovarian Follicles in Dairy Cattle: a Review. Reprod. Nutr. De., 46, 105-119. Veerkamp, R.F.; Oldenbroek, J.K.; VanDer, Gaast, H.J.; Van Der, Werf, J.H. J. (2000). The genetic correlation between days until start of luteal activity and milk yield, energy balance and live weight. J. Dairy. Sci, 83, 577-583. Vet. Clin. North Am. Food. Anim. Pract. 8:29–56. 23.