THE EFFICACY OF INTRA-UTERINE INFUSION OF IODINE COMPOUNDS ON THE REPRODUCTIVE EFFICIENCY OF POSTPARTUM AND REPEAT BREEDER CROSS-BRED DAIRY COWS IN THE SUDAN

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A thesis submitted in fulfillment of the requirements of University of Khartoum for Doctor of Philosophy in Veterinary Medicine

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May (2008)

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DEDICATION

To the soul of my uncle Elfadil Ahmed Elzubeir and to the lovely eyes of my kids Omer and Tarig I dedicated this thesis.

ABBREVIATIONS

BCS: Body condition score
CI: Calving interval
DF: Dominant follicle
DO: Day open
EB: Energy balance
FO: First oestrus
PP: Postpartum
PPP: Postpartum period

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AKNOWLEDGEENTS

First of all thanks to Allah for providing me with health and strength to perform this study.

I am greatly indebted to my supervisor A. S. Elsheikh for suggesting the topic and his close supervision, encouragements and keen guidance of my applied research.

I am greatly indebted to Dr A. S. Ali Department of Preventive Medicine and Public Health, Faculty of Veterinary Medicine, U. of K. for his proper assistance and friendly attitude.

My utmost appreciation and gratitude are reserved to Prof A. M. Salih, Department of Animal Nutrition, Faculty of Animal Production, U. of K. for his advice, interest in my research and fatherly attitude. I acknowledge with gratitude to the technical staff at the Department of Microbiology, Faculty of Veterinary Medicine, U. of K. for their kind help and availing every possible facilities to bring this study into reality.

My special thanks are also due to the staff of U. of K. dairy farm at which this study was carried out.

Finally I would like to thank all those who contributed to this study in one way or anther.

ABSTRACT

This study was conducted to determine the percentage of uterine bacterial infection among PP cross-bred dairy cows and to evaluate its influence on their reproductive efficiency. Furthermore, the effects of intra-uterine infusion of iodine compounds (1% Lugol's iodine and 2% Povidone iodine) on the reproductive efficiency of PP and repeat breeder dairy cows was investigated.

Experiment I. was designed to determine the incidence of uterine bacterial infection among early PP dairy cows. Furthermore, the effects of uterine bacterial infection on PP reproductive efficiency of the same cows were evaluated by studying six reproductive parameters. These parameters were uterine involution, appearance of the first DF, appearance of the FO, length of DO, rate of service per conception and CI. Uterine endometrium swabs were collected on day 5 PP from 130 dairy cows and were cultured within 2 hours of collection in blood agar media and MacConkey agar media. The uteri of 120 cows (93%) were found infected and 10 cows (7%) were found none infected. From the total infected cows 40 cows were used to evaluate the effect of bacterial uterine infection on their reproductive performance. Twenty cows were severely infected and the remaining cows (20 cows) were mildly infected. The result of this experiment showed that dairy cows that suffered sever uterine bacterial infection had a significantly ($P<0.001$) extended uterine involution period, long time for the appearance of the DF and the FO, the length of DO and CI compared to the dairy cows that suffered mild uterine bacterial infection. Moreover, the dairy cows which suffered sever uterine bacterial infection had a significantly ($P<0.001$) increased rate of

service per conception compared to the cows with mild uterine bacterial infection.

Experiment II. This experiment was conducted to study the effects of intra-uterine infusion of diluted Lugol's iodine on PP reproductive efficiency of cows having uterine bacterial infection. From the remaining cows that were diagnosed as severely infected in experiment **I,** 40 cows having severe uterine bacterial infection were selected. The cows divided randomly into two equal groups (A) and (B) 20 cow each. Group A was intra-uterine infused with 1% Lugol's iodine on day 5 PP. Group B was left untreated to serve as a control. The above mentioned parameters were evaluated. The result of this experiment showed that infusion of 1% Lugol's iodine significantly improved ($P<0.0001$) all the reproductive parameters compared to the control.

Experiment III. This experiment was conducted to study the effects of intra-uterine infusion of diluted Povidone iodine on PP reproductive efficiency of cows having uterine bacterial infection. From the remaining cows that were diagnosed as severely infected in experiment **I,** 40 cows having severe uterine bacterial infection were selected. The cows were divided randomly into two equal groups (A) and (B) 20 cow each. Group A was intra-uterine infused with Povidone iodine on day 5 PP. Group B was left untreated to serve as control. The above mentioned parameters were evaluated. The result of this experiment showed that infusion of 2% Povidone iodine significantly improved ($P<0.0001$) all the reproductive parameters compared to the control.

Experiment IV. was conducted to study the possibility of using diluted Lugol's iodine for treating repeat breeder cow syndrome. Sixty cross-bred repeat breeder dairy cows were used in this experiment.

Uterine swabs were collected from uteri of all cows. After bacterial culture all the selected cows were found mildly infected. They were then grouped randomly into three groups. Group A (n = 20 cows) was intra-uterine infused with 1% Lugol's iodine 6 hours pre-insemination. Group B (n = 20 cows) was infused with the same solution 6 hours post-insemination. Group C (n = 20 cows) was left without treatment to serve as control. The potency of diluted Lugol's iodine to cure repeat breeder cow syndrome was evaluated by studying the DO, rate of service per conception and the CI. The results of this experiment showed that, the DO, rate of service per conception and CI were significantly (P<0.001) reduced by infusion of diluted Lugol's solution 6 hours pre/post insemination as compared to the control.

Experiment V. was conducted to study the possibility of using diluted Povidone iodine for treating repeat breeder cow syndrome. Sixty cross-bred repeat breeder dairy cows were used in this experiment. Uterine swabs were collected from uteri of all cows. After bacterial culture all the selected cows were found mildly infected. They were then grouped randomly into three groups. Group A (n = 20 cows) was intra-uterine infused with 2% Povidone iodine 6 hours pre-insemination. Group B (n = 20 cows) was infused with the same solution 6 hours post-insemination. Group C (n = 20 cows) was left untreated to serve as control. The potency of diluted Povidone iodine to cure repeat breeder cow syndrome was evaluated by studying the same parameters as in experiment 4. The findings of this experiment showed that, the infusion of diluted Povidone iodine pre/post insemination significantly (P<0.001) reduced the DO and CI as compared to the control. Furthermore, the rate of service per conception was significantly (P<0.001) minimized compared to the control.

It is concluded that, the reduced reproductive efficiency of cross-bred dairy cows is likely to be due to early PP uterine bacterial mild infection that decreases reproductive efficiency and leads to repeat breeding syndrome later. Intra-uterine infusion of 1% Lugol's iodine can be used successfully to improve the PP reproductive performance of cross-bred dairy cows.

موجز الأطروحة

أجريت هذه الدراسة لتحديد نسبة الاصابه ببكتريا الرحم بين أبقار الحليب الهجين ولتقييم الآثار التي تحدثها هذه الإصابه البكتيرية على كفاءتها التناسلية. وكـذلك لمعرفـة الآثار التي يحدثها تسريب الرحم بمحاليل اليود (محلول اللوقـول المخفـف و ايـودين البوفيدون المخفف) على الكفاءة التناسلية للابقار حديثة الولاده و الأبقار مرتدة الشبق.

التجربة الأولى: أجريت هذه التجربه لتحديد نسبة الاصابه ببكتريا الرحم بين أبقار الحليب الهجين بعد الولاده و لدراسة الآثار التي تحدثها هذه الإصابه البكتيريه عقب الولادة علـى كفاءتها التناسلية والتي تم تقييمها بدراسة المعالم التاليه أوب الرحم, ظهور أول حويـصلة جراف سائدة , ظهور أول دورة شبق, الفتره المفتوحه, معدل التلقيحات اللازمه لأحـداث الحمل والفتره ما بين الولادتين. أخذت مسحات رحميه في اليوم الخامس بعد الولادة من 130 بقره حليب هجين للتزريع البكتيري. تم تزريع المسحات بعد ساعتين من جمعها في وسط أجار الدم ثم و سط أجارماكونكى فوجد أن عدد الأبقار التي تعاني مـن اصـابات بكتيرية في الرحم عددها 120 بقره" (93%) بينما السليمة عددها 10 ابقار (7%). تـم اختيار 40 بقره من الابقار المصابه لدراسة أثر التهابات الرحم البكتيريه علـى الكفـاءة التناسلية لهذه اللأبقار بعد الولاده. قسمت اللأبقار الي مجموعتين حسب النمو البكتيـري. مجموعه مكونه من 20 بقره تعانى من اصابات رحميه بكتيرية شديدة و مجموعه مكونه من 20 بقره تعانى من اصابات رحميه بكتيرية خفيفة. .أظهرت نتائج هذه التجربـة ان إصابات الرحم البكتيرية الشديده بعد الولادة لها تأثير معنـوي ($P<0.001$) فـي إطالـة الزمن المطلوب لأوب الرحم , ظهور أول جريب جراف سائد ، ظهور أول دورة شـبق ,الفترة المفتوحة و الفتره مابين الولادتين. إضافة إلى ذلك فإن إصابات الرحم الشديده بعد الولادة فى الابقار الحليب تزيد من معدل التلقيحات اللازمة لإحداث الحمـل ($P<0.001$) مقارنة بالابقار ذات الإصابة البكتيرية الخفيفة.

التجربة الثانية: أجريت هذه التجربه لدراسة الآثار التي يحدثها تسريب الـرحم بمحلـول اللوقول المخفف (1%)فى اليوم الخامس بعد الولاده على الكفاءة التناسـلية للابقـارالتي تعاني من اصابات رحم بكتيريه. تم استخدام40 بقرة حليب هجين تعاني من اصـابات رحميه شديده من أبقار التجربه الاولي وتم تقسيمها عشواءيا لمجمـوعتين (أ) و (ب) 20 بقره لكل. تم تسريب الرحم بمحلول اللوقول المخفف لابقار المجموعـه (أ). المجموعـة (ب) اتخذت كمجموعة شاهد. ثم تم تقييم المعالم السالفه . أظهرت نتائج هذه التجربـة

أن تسريب الرحم بمحلول اللوقول المخفف له أثر معنوي (P<0.0001) في تحسين كافة المعايير التناسليه التي درست عند مقارنتها بمجموعة الشاهد.

التجربة الثالثه: أجريت هذه التجربه لتقييم الآثار التي يحدثها تـسريب الـ رحم بمحلـ ول أيودين البوفيدون المخفف (2%) فى اليوم الخامس بعد الولاده علـى الكفـاءة التناسـلية للأبقارالتي تعاني من اصابات رحم بكتيريه. تم استخدام 40 بقرة حليب هجين تعاني من اصابات رحميه شديده من أبقار التجربه الأولي وتم تقسيمها عشواءيا لمجمـوعتين (أ) و (ب) 20 بقره لكل. تم تسريب الرحم بايودين البوفيدون المخفف لأبقار المجموعـه (أ). المجموعة (ب) اتخذت كمجموعة شاهد. ثم تم تقييم المعالم السالفه . أظهـرت نتـائج هذه التجربة أن تسريب الرحم بايودين بالبوفيدون المخفف له أثر معنوي (P<0.0001) في تحسين كافة المعايير التناسليه التي درست عند مقارنتها بمجموعة الشاهد.

التجربة الرابعة: أجريت هذه التجربة لدراسة إمكانية استخدام محلول اللوقول المخفـف (1%) في علاج ظاهرة البقرة مرتدة الشبق.استخدمت 60 بقرة حليب هجين مرتدة الشبق لدراسة الآثار التي يحدثها تسريب الرحم بمحلول اللوقول المخفف. أخذت مسحات رحميه للتزريع البكتيري فوجد أن جميع الابقار تعانى من اصابات رحم بكتيرية خفيفه. تم تقسيم الأبقار عشوائياً إلى ثلاثة مجموعات (أ) , (ب) , (ج) فـي كـل مجموعـه 20 بقـرة. المجموعة (أ) تم علاجها بتسريب الرحم بمحلول اللوقول المخفـف قبـل التلقـيح ب 6 ساعات والمجموعه (ب) تم علاجها بتسريب الرحم بنفس المحلـول بعـد التلقـيح ب 6 ساعات والمجموعة (ج) اتخذت كمجموعة شاهد. فعالية محلول اللوقول المخفف في علاج ظاهرة البقرة مرتدة الشبق تم تقييمه من خلال تاثيره علـى الفتـرة المفتوحـة و معـدل التلقيحات اللازمه لأحداث الحمل و الفتره بين الولادات .أظهرت نتائج هذه التجربـة أن تسريب الرحم بمحاول اللوقول المخفف قبل التلقـيح أو بعـد التلقـيح يقـصر معنويـا (P<0.001) الفترة المفتوحة و الفترة بين الولادتين كما أنه يقال من معـدل التلقيحـات اللازمة لإحداث الحمل إذا ماقورنت بمجموعة الشاهد (P<0.001).

التجربة الخامسة: أجريت هذه التجربة لدراسة إمكانية استخدام محلول أيودين البوفيـدون المخفف(2%) فى علاج ظاهرة البقرة مرتدة الشبق . استخدمت 60 بقرة حليب هجـين مرتدة الشبق لدراسة هذه الآثار التي يحدثها أيودين البوفيدون المخفف. تم تقسيم الأبقار عشوائياً إلى ثلاثة مجموعات (أ) , (ب) , (ج) في كل واحدة 20 بقرة ثم أخذت مـسحات رحميه من هذه الأبقار للتزريع البكتيري فوجد أن جميع الأبقار تعانى من إصابات رحـم بكتيرية خفيفه. المجموعه (أ) تم علاجها بتسريب الرحم بأيودين البوفيدون المخفف قبـل التلقيح ب 6 ساعات والمجموعه (ب) تم علاجها بتسريب الرحم بنفس المحلول بعد التلقيح

ب 6 ساعات والمجموعة (ج) اتخذت كمجموعة شاهد. فعالية ايودين البوفيدون المخفف في علاج ظاهرة البقرة المرتدة الشبق تم تقييمها من خلال تأثيره على المعالم السابقة فى التجربه الرابعه. أظهرت نتائج هذه التجربة أن تسريب الرحم بايودين البوفيدون المخفف قبل أو بعد التلقيح يقصر معنويا (P<0.001) الفترة المفتوحة و الفترة بين الولادتين كما يقلل من معدل التلقيحات اللازمة لإحداث الحمل إذا ما قورنت بمجموعة الشاهد (P<0.001).

خلصت هذه الدراسة الي أن تدني الكفاءة التناسلية لأبقار الهجين يعزى بشكل أساسي الى إصابات الرحم البكتيرية الخفيفه بعد الولادة والتي تؤدي فى مجملها لظاهرة البقرة مرتدة الشبق في نهاية المطاف. وأن تسريب الرحم بمحلول اللوقول أو بأيودين البوفيدون المخفف يساهم بفعالية ونجاح فى تحسين الكفاءه التناسليه لهذه الابقار.

INTRODUCTION

During the past few decades, dramatic increase in infertility and reproductive disorders associated with a rising in milk yield in dairy cows was reported. This could be attributed to genetic selection for milk yield, improved nutrition as well as new management practices (Jolly *et al*; 1995).

Concerning the image of dairy farm in the Sudan, Elsheikh and Ahmed (2004) reported that, the cross-bred dairy cows in the Sudan have a long postpartum period (PPP) to first oestrus, which affects their reproductive efficiency. In dairy cows, numerous ovarian follicular waves resume soon after parturition (Yavas and Walton, 2000). The first postpartum (PP) follicular wave can be detected 10 days after parturition in dairy cows (Stagg *et al.,* 1995) and the incidence of ovulation of the first dominant follicle (DF) is low (Savio *et al*., 1990). The delayed commencement of the PP ovarian activity in cross-bred dairy cows is associated with increasing genetic merits for milk yield, lack of sound nutrition system, heat stress, endometritis and mismanagement (Dobson and Smith, 2000). The fate of the PPDF fell into one of three options: a) In 42% of PP dairy cows, DF of the first PP follicular wave ovulates b) In 40% of PP dairy cows, the first PPDF regresses c) in 18% of PP dairy cows it becomes cyst (Beam and Butler, 1997). The induction of oestrus cycles following calving has been the stimulus for much reproductive research efforts over the past decades (Short et al., 1999; Elsheikh and Ahmed, 2004; Elsheikh and Ahmed, 2005). The extended PP a cyclicity is attributed to the absence of appropriate LH pulses, which lead to artesia of follicles that developed PP (Yavas and Walton, 2000). During early PP, pituitary LH stores are depleted (Nett, 1987; Nett *et al*., 1987), but a round the third week PP, LH stores are replenished (Yavas and Walton; 2000). The prolonged PPI in dairy cows is due to failure of ovulation of the DF rather than delayed development of them and this is the major problem that limits the improvement of reproductive efficiency of dairy cows (Short *et al*., 1999; William and Griffith, 1995). Ovulation failure of DF during early PP was associated with production of milk fat (4%) and a delayed energy balance (EB) nadir, where EB may be an important regulator in resumption of PP cyclic activity in dairy cows (Beam and Butler, 1997). Moreover, delays in the resumption of ovulation during early PP is associated with repeat breeding which increase the calving intervals (Rhodes *et al*., 2001). The injection of GnRH or $PGF_{2\alpha}$ during early PP accelerates recrudescence of ovarian activity in dairy cows (Elsheikh and Ahmed, 2005). The reduction in the time taken for recrudescence of oestrus PP in dairy cows treated with GnRH or $PGF_{2\alpha}$ was attributed to initiation and synchronization of follicular wave (Elsheikh and Ahmed, 2004; Ahmed and Elsheikh, 20004; Elsheikh and Ahmed, 2005).

Over the last 10 years, intra-uterine infusion with diluted iodine compounds such as 1% Lugol`s and 2% Povidone iodine is used to improve the reproductive performance of PP and repeat breeder dairy cows (Geiser, *et al.,* 1995). Moreover, intra-uterine infusion of diluted Lugol's (1%) or diluted Povidone (2%) iodine is an efficient treatment which improves the PP reproductive performance and increases the conception rate even in repeat breeder cows (Koujan *et al*., 1996). In the Sudan neither 1% Lugol’s nor 2% Povidone iodine are used for management of PP reproductive problems or repeat breeder syndrome in dairy cows. In view of the above mentioned findings.

The main objectives are:

1. To determine the percentage of the cross-bred dairy cows having uterine bacterial infection during early PP.
2. To determine the effects of uterine bacterial infection during early PP on uterine involution, appearance of the first DF, recrudescence of the FO, DO, rate of service per conception and calving interval (CI) in cross-bred dairy cows in the Sudan.
3. To determine the effects of intra-uterine infusion of diluted Lugol's iodine (1%) or diluted Povidone iodine (2%) on day 5 PP on the same parameters mentioned above.
4. To determine the effects of intra-uterine infusion of diluted iodine compounds on the DO, rate of service per conception and CI in repeat breeder cross-bred Sudanese dairy cows. .

CHAPTER ONE
LITERATURE REVIEW

I. Postpartum period (PPP) in dairy cows

1.1. Definition

It is the time from parturition to complete involution of the uterus and it could be as short as 15 days or longer than 100 days (Malven, 1984., Olson, *et al*., 1986). The PPP is divided into three periods, the puerperal period which is the period from parturition until the pituitary becomes responsive to GnRH (7 to 14 days), the intermediate period which begins after the increment of pituitary sense to GnRH and ends after the first PP ovulation (14 to 20 days) and the post-ovulatory period which is the time from the first PP ovulation until uterine involution is completed (Macmillan and Thatcher, 1991). The PPP depends on the level of nutrition, body condition score (BCS), suckling, milk yield, age, season, breed, mastitis, heat stress, management and uterine bacterial infection (Short *et al*., 1999 and Stagger *et al*., 1998). The recrudescence of follicular growth occurs soon after calving (Savio *et al*., 1990). The PP first ovulation in dairy cows occurs 3 to 5 weeks and it is related to EB during the first 3 weeks (Grummer and Carroll, 1991). The ovaries rebound within 30 to 102 days PP in the dairy cows (Beam and Butler., 1997: Toribio *et al*., 1995). Nearly about 90% of dairy cows start oestrus cycles by 60 days PP, but only about 60% were detected correctly in oestrus at that time (Younquist, 1988).

1.2 Postpartum (PP) follicular wave patterns in dairy cows

1.2.1 Definition

Ovarian follicular wave is the emergence of a DF or medium-DF and a number of smaller follicles that apparently originate from the same follicular pool (Savio *et al*., 1988). The most frequent patterns of follicular development in dairy cow are the two follicular waves pattern (Fortune, 1993) and the three follicular waves pattern (Toribio, *et al*., 1995). Cycles with one or four follicular wave's pattern are reported less frequently (Carrier, *et al*., 1994). The occurrence of two or three waves is linked to differences in fertility (Savio, *et al*., 1988). Dairy cows with three waves of follicles are reported to have higher conception rates (Ahmed, *et al*., 1997). In dairy cows, numerous ovarian follicular waves resume soon after parturition. However, no ovulation occurs (Yavas and Walton, 2000). The follicular waves in dairy cows resume during the first week PP for the two-waves, but for the three follicular waves they resume during the second week PP (Ginther, *et al*., 1989). Inhibitory and stimulatory ovarian factors play an important role in follicular development through the regulation of both FSH and LH and the response of the follicles to these gonadotropins (Ireland, 1997: Toneta and de Zerga, 1989). Although early development of follicles is independent of gonadotropins, little is known about the factors that initiate follicular growth at these stages. A primary follicle takes many weeks to reach ovulatory size (Britt, 1992). Therefore, follicles that ovulate I to 3 months PP initiate early development during the period of sever negative EB. The cow ovaries contain hundreds of small and primary follicles, but at any given time, a small number of primary follicles begin to develop and differentiate into secondary and tertiary follicles (Duffy, *et al*., 2000). The same authors attributed the prolonged PPP in dairy cows to failure of early DF to ovulate. This failure of ovulation is hypothesized to be due to inadequate LH pulse frequency. The average oestrus period of dairy cows is 21 days (Alves, *et al*., 2002). The same authors speculated that, interovulatory period in the three wave's pattern cycles is 24 days which is relatively longer when it is compared with the one wave pattern cycle (16 days) and the two wave's pattern cycles (20 days). The same authors emphasized that, the follicular wave average length are 11, 8 and 7 days receptively, for the first, second and third follicular wave.

1.2.2 The fate of postpartum dominant follicle (PPDF)

The DF is the largest follicle that appears in the ovary and suppresses the growth of the other follicles and grows linearly for approximately 6 days until it reaches (1mm) and begins to regress (Murphy, *et al*., 1995). The first PPDF is formed as early as 10 days and ovulates in more than 70% of dairy cows (Savio, *et al.,* 1990). Contrary, in dairy cows with a short PPI there is sequential emergence of medium follicles within 7 to 14 days of calving from which a single follicle is selected to become DF (Murphy, *et al*, 1995; Savio, *et al.,* 1990). The first PPDF ovulates, undergoes atresia or becomes cystic. The prolonged PP anoestrus in dairy cows is due to failure of ovulation rather than delayed development of the DF (Wael and Noseir, 2003). The same authors documented that ovulatory follicle is always greater and produce higher estradiol. Physiologically, the DF is postulated to secrete factors, including inhibin, activin and follistatin, which regulate the growth of other follicles (Ireland, 1987). The gonadotropins regulate the follicular growth and differentiation of the exact mechanisms which determine dominance, but onset of atresia is still unknown (Tonetta and de Zerga, 1989). The first PP follicular wave development in dairy cattle provides a unique model for the study of follicular dominance and the DF of this wave is identified as early as 5 days in the cycle (Sirois and Fortune, 1988). There are recurrent waves of follicular growth in most physiological states in cattle (Roche, *et al.,* 1991). Moreover, they stated that the duration of a wave from follicular emergence to its ovulation or its atresia is 7 to 8 days. Additionally, Beam and Butler (1997) reviewed that, a wave of follicular development is formed during the first week PP and it is characterized by emergence of a large DF. They also reported that, the fate of the first PPDF fell into one of three options, in about 42% of the dairy cows, the first PPDF ovulates, in about 40% of the dairy cows, first PPDF regresses and in about 18% of the cows it persist and it develops into a cyst. The same authors found that, the DF in ovulating dairy cows produce more estrogen and reached a maximum diameter than the DF in nonovulatory dairy cows. As follicles grow, they are more likely to become artretic and only DF is normally expected to complete maturation (Ko, *et al.,* 1991). Several studies on follicular development and viability, based on steroid secretion, indicated that most medium and large follicles become artretic (Skyer, *et al*., 1987). Furthermore, in all follicular waves that occur during the luteal phase of anoestrus cycle or in pregnancy all the follicles including the DF become artretic (Ko, *et al*., 1991). The presence of a functional CL inhibits the secretory patterns of gonadotropins that allow the final maturation of the DF (Roche. *et al.,* 1991 and Fortune, 1993). These differences in the fate of the DF delay the resumption of ovulation and expression of estrus during early PP in dairy cows. Thus, a reduced conception rate and an elevated CI were recorded (Rhodes, *et al*., 1999).

1.2.3 Factors affecting postpartum (PP) follicular wave patterns

1.2.3.1 Hormonal influences

FSH

The level of FSH is reported to increase from low concentration in prepartum to higher levels within the first 3 to 5 days PP (Lamming, *et al*., 1981; Peters and Horst, 1981). The high FSH level is essential for initiation of ovarian activity during the PP (Peters and lamming, 1984). The same authors concluded that, at or just after ovulation in dairy cows, there is a transient 1 to 2 day's rise in FSH, which is associated with the emergence of 5 to 7 mm follicles on days 1 to 2 of the cycle. These follicles continue to grow and produce oestardiol that suppresses FSH secretion. Thus, FSH decline to baseline or nadir concentrations by day 4 of the cycle. When the follicles are exposed to low FSH concentrations, a single follicle is selected to grow, while the others cease (Ginther, *et al.,* 1996). Although FSH is essential for follicular development, its lack is not a major factor that delays the PP recrudescence of folliculgenesis and even ovulation in dairy cows (Xu, *et al*., 2000, Sterry, *et al*., 2007).

LH

Initially, pituitary LH content and responsiveness to GnRH increases rapidly from their nadir at parturition in dairy cows (Schallenberger, *et al*., 1984). Recovery of the pituitary seems nearly to be completed by day 10 in milked dairy cows, but basal plasma LH concentration rises as more frequent episodic pattern of pulstile LH is released (Moss, *et al*., 1985). This pulse pattern appears to be the primary stimulus for ovarian follicular development that leads to first ovulation (Lamming *et al.,* 1981). The re-establishment of a normal LH pulse pattern is the key factor responsible for ovarian follicular development and the initiation of PP ovarian cyclicity (Butler and Smith, 1989). In dairy cows LH seems to be deficient after 5 to 10 days PP (Schallenberger, *et al*., 1984). The infrequent LH pulses during early PP lead to atresia rather than ovulation of the DF (Yavas and Walton, 2000). The main factors that delay the increase in LH pulse frequency are suckling, lower energy intake, inadequate body reserves, high milk production, heat stress and peripartum diseases (Rhodes, *et al.,* 1998). Low LH pulse frequency during the luteal phase prevents the occurrence of maturation and ovulation of the DF (Roche. *et al.,* 1991). The DF becomes dependent on LH and its fate is determined by LH pulse frequency (Rhodes, *et al*., 2003). In dairy cows, when the DF is exposed to LH pulse of 3.5 to 4.5 ng/m1/6 hrs, it ovulates. However, it becomes atretic if it is exposed to LH level of 1.7 to 2.2 ng/m1/6 hrs or becomes cystic ovary if exposed to very low LH concentrations (Beam and Butler, 1997). In dairy cows, LH concentration remains low until the EB nadir is reached (Canfield and Butler, 1990). When the EB begins to move in a positive direction, LH begins to increase and ovulation occurs (Rhodes, *et al*., 2003).

Oestrogens

The level of oestrogen increases from day 26 prepartum and then decreases during the first week PP (Smith, *et al.,* 2003: Bo, *et al.,* 1995: Haughian, *et al.,* 2002). The oestrogen increment triggers a positive feed back mechanism on the hypothalamus and anterior pituitary gland that enhances preovulatory LH surge (Arthur, *et al.,* 1998). Oestradiol concentration, which depends mainly on follicular size, plays an important role in control of follicular development (Wael and Noseir, 2003).

Progesterone

After the end of oestrus phase, the ruptured follicle forms the CL and progesterone concentrations increase gradually over the next few days (Rhodes, *et al.,* 2003). The progesterone is the key hormone that regulates the duration of the oestrus cycle and is slowly secreted by the CL. The progesterone experts negative feed back effects on gonadotropin releasing hormone (GnRH). Consequently, LH pulse frequency decreases from 1 ng/ml in the follicular phase to 1 ng/ml every 2 to 4 hours in the luteal phase (Roche. *et al.,* 1991). Contrary, low concentration of progesterone causes increment of LH pulse frequency (Fortune, 1993). The plasma concentration of progesterone hormone is high during last stage of pregnancy and then begins to drop in concentration before parturition and remains very low over the first 4 to 5 days PP (Arthur, *et al.,* 1998). The sudden drop of progesterone following parturition lead to removal of the negative feed back on the gonadotropins and thus exerts a positive feed back upon the hypothalamus to release GnRH which stimulates the anterior pituitary to release FSH and LH (Savio, *et al.,* 1990). The progesterone concentration during early PP has no effect on the interval from new wave emergence to dominance (Ryan, *et al*., 1998). For instance, progesterone decreases the muscular activity of the uterus and stimulates the secretion of uterine milk from epithelial cells lining the uterus that improves the life-hood of embryo growth (Oakley, 1992).

Oxytocin

The release of oxytocin hormone is stimulated by sensory receptors in the cervix and anterior vagina (Rhodes, *et al.,* 2003). The level of oxytocin reaches its maximum level (6.4 Pg/ ml) at the expulsive stage of parturition and falls to the minimum level (0.5 pg/ ml) at time of oestrus (Hafez, 1993). The oxytocin level during last pregnancy and early stages of parturition remains low and increases to reach the peak values when the fetal head emerges from the vulva. The uterus is a target reproductive organ for oxytocin. It causes contraction of the smooth muscles in the uterine wall, thus assist in the emptying of the uterus when the cervix is open, increasing uterine involution and ovulation of the first PPDF (Arthur, *et al.,* 1998). Oxytocin is much more effective when the uterus is under control of estrogen (Roberts, *et al*., 1987).

Prostaglandin $F_{2\alpha}$

During early PP, the uterus produces and metabolizes large amount of $PGF_{2\alpha}$ (Guilbault, *et al*., 1984). In dairy cows, the $PGF_{2\alpha}$ increases after the end of parturition to reach peak values 3 days PP and does not return to basal levels until 15 day (Edquvist, *et al.,* 1980). Administration of $PGF_{2\alpha}$ during early PP reduced the time taken for uterine involution, accelerated recrudescence of the first PP oestrus, reduced the DO, CI and improved conception rate in cross-bred dairy cows (Michiel, 1999: Schofield, *et al.,* 1999, Elsheikh and Ahmed, 2004). Treatment with the $PGF_{2\alpha}$ during early PP once or twice at 10 to 14 day's interval decreases the DO (Yougquist, 1988). Sequential treatments with the $PGF_{2\alpha}$ (days 21, 34 and 57) PP stimulated early cyclicity in dairy cows (Zemjanis, 1980 and Risco, *et al.,* 1995). The uterus produces high concentrations of $PGF_{2\alpha}$ during the early PPP. These prostaglandins play an important role in uterine involution (Younquist, 1988; Salam, *et al.,* 1994). When the exogenous $PGF_{2\alpha}$ were used twice daily for 10 days started from 3 days PP, the uterine involution was accelerated by 6 to 13 days (Kindahl, *et al*., 1982). Treatment with $PGF_{2\alpha}$ between 14 to 28 days PP enhances uterine involution and increased conception rate (Young, *et al.,* 1984). In dairy cows, PP prostaglandin levels are influenced by stress during parturition (dystocia, transportation) as well as by retained placenta (Heuwieser*, et al.,* 1992).

Prolactin

Prolactin increases 2 to 4 days before parturition and continues to increase after parturition in both milking and suckling dairy cows (Hafez, 1993). Suckling and milking had no effect on prolactin levels (Smith, *et al.,* 1987). The release of prolactin is under the control of prolactin inhibitory factor, which is released from the hypothalamus (Arthur, *et al.,* 1998).

Insulin:

Insulin has gonadotropic effects on follicular development (Butler and Smith, 1989). Insulin concentration rather than glucose may directly affect LH secretion (Spicer*, et al*., 1993). Moreover, insulin is largely responsible for regulation of LH pulse patterns that seems to be crucial for the onset and timing of PP ovarian function. High insulin concentration in diet increased the proportion of dairy cows that ovulate 50 days PP and reduced the intervals from calving to first ovulation (Gong, *et al.,* 2002). This because insulin is a potent stimulator of both proliferation and function of follicular cells (Spicer, *et al.,* 1993). The same authors stated that IGF-1 had stimulatory effects similar to insulin. Therefore, one may extrapolate that optimal follicular development only occurs in the presence of adequate concentrations of insulin and/or IGF-1. However, both of the above mentioned compounds are low during the PP period (Beam and Butler, 1998). The same authors found that, the development of ovulatory follicle is related to insulin and IGF-1 concentrations in PP dairy cows. Moreover, higher concentrations of plasma IGF-1 are associated with earlier occurrence of CL development and total progesterone concentrations during PP in dairy cows (Thatcher, *et al.,* 1996). Insulin and/or IGF-1 are necessary for maximal responsiveness of follicles to the stimulatory effects of FSH and LH (Lucy, *et al.,* 1992). Moreover, follicular competence during early PP in dairy cows is associated with higher plasma IGF-1 (Beam and Butler, 1997).

Corticosteroids

During the final stages of gestation, the plasma concentration of cortisol increases (Dobson and Smith, 2000). Due to the rapid growth of the fetus and increasing the demand to the metabolic agents this stimulates the placenta to produce $PGF_{2\alpha}$, which stimulates the fetal hypothalamus-pituitary and adrenal axis leading to increase concentration of cortisol (Hafez, 1993). The cortisol stimulates the placenta to convert progesterone to oestrogen. The elevated level of oestrogen stimulates secretion of $PGF_{2\alpha}$ from uterine wall and enhances developing of oxytocin receptors.

Iodine

Iodine is a trace mineral essential for normal growth and development and 70% to 60% of iodine in the body is found in the thyroid gland (Yu and Tak-yin, 1993). The physiological regulation of thyroid gland by iodine is complex and involves feedback mechanism at several biochemical and physiological steps that depend on the amount of iodine and route of administration (Sarkar, 2006). Thyroid hormones control iodine movement throughout the body (Obaidullah, 1981). Also the same authors reported that hypothyroidism caused by iodine deficiency in dairy cattle consequently reduces fertility. Furthermore, he found that iodine offers a variety of potential therapeutic uses primarily in the prevention and treatment of hypothyroidism caused by iodine deficiency. Sudden large doses of iodine impair the production of thyroid hormones, which cause hypothyroidism temporarily. In soil with low iodine, infertility is estimated to be due do endocrine disorders that are caused by iodine deficiencies (Gotherdi, 1991). Iodine is an essential nutrient for optimal thyroid function in dairy cattle (Sarkar, 2006). Reproduction in dairy cows is influenced through iodine's action on the thyroid gland. Thus, improved follicular development, ovulation and maintenance of pregnancy in dairy cattle are a function of adequate thyroid activity (Bailey, *et al.,* 1999). The same author reported that iodine supplementation is recommended, whenever necessary, to insure that cows receive enough iodine each day to increase the function of thyroid gland, maintains the balance of calcium, increases metabolic activity of the body and the animal displays heat early. It is interesting to note that different classes of microorganisms vary in their susceptibility to iodine where bacteria are very sensitive, viruses are intermediate, but protozoa cysts are more resistant. Doses of iodine below 1 ng/ml are effective for bacteria within minutes (Sarkar, 2006).

1.2.3.2 Suckling

The suckling is the major factor that affects duration of the PP ovarian activity in dairy cows (Xu, *et al.,* 2000). Continuous suckling extends the interval from parturition to the first oestrus and ovulation (Mukasa, *et al.,* 1991). The suckling interferes with hypothalamic release of GnRH and provokes a marked suppression on pulstile LH release, which extends PP anoestrus in dairy cows (Short, *et al*., 1999; Montiel and Ahuja, 2005). Contrarily, restriction of suckling to once-daily or complete isolation of calf from suckling dairy cows promotes an earlier ovulation through improvement of nutritional status compared to continuous suckling in dairy cows (Patton, *et al.,* 2006). During early lactation and suckling plasma growth hormone (GH) and IGF-1 concentrations are reported to be high (Bar-Peled, *et al.,* 1995). Similarly, the same group reported a high increment in the level of prolactin and oxytocin, but they reported a decrement in insulin level. Conclusively, the maternal bond is the major factor that delays resumption of ovulation and it is dependent upon visual and/or olfactory signals between dam and calf (Stagger, *et al.,* 1998; Williams and Griffith, 1995). Thus, temporary calf removal is effective for reduction of PPP to first ovulation in dairy cows (Stagger, *et al.,* 1998; Mackey, *et al*., 2000; Rhodes, *et al.,* 2003).

1.2.3.3 Milk yield

High milk yield is known to increase the length of PPP to first oestrus and calving to conception (Gutievres, *et al.,* 1999; Gong, *et al*., 2002). This is definitely due to the high metabolic demand during high lactation (Srejerson and Neimmann, 1982). Therefore, the influences of high milk yield on reproductive performance during early PP could not be separated from nutrition (King, 1968). Slight antagonism between milk yield and the reproductive performance was reported (Fonesca, *et al.,* 1983). This is due to the stress associated with high milk yield during the early PP (Walters, *et al.,* 2002). When the milk yield increases, the reproductive performance decreases. High milk producers resume PP ovarian activity at about the same time as medium milk producers (Harrison, *et al.,* 1990). This correlation between milk yield and the time span to first ovulation becomes significant only at 40 days in dairy cows (Butler and Cappock, 1981).

1.2.3.4 Mastitis

. The occurrence of mastitis during early PP in dairy breed cows increases the DO and services per conception as compared to uninfected cows ((Fetrow, *et al.,* 1991: Santos, *et al.,* 2004). Dairy cows suffering from mastitis during the first month PP show a delay in the onset of ovarian activity (Huszenicza, *et al.,* 2005). Mastitis during early PP triggers the adrenal cortex to secrete cortisol, which suppresses the hypothalamus-pituitary-ovarian axis to release FSH and LH (Rhodes, *et al.,* 2003). Consequently, decreases follicle development, maturation of the oocye and ovulation rate. The dairy cows with clinical mastitis during early lactation exhibit a prolonged PPP (94 days) than the dairy cows without clinical mastitis (71 days) (Baker, *et al.,* 1998). Sub-clinical mastitis during early PP also reduces the reproductive efficiency of dairy cows (Moore and Oconnor, 1993). In lactating dairy cows, mastitis due to bacterial infection with Gram-positive and Gram-negative pathogens has similar effects on increasing DO, and the service per conception (Hockett, et *al.,* 2000).

1.2.3.5. Season

The season influences the duration of the PPI in both suckled and nonsuckled dairy cattle (Lamming, *et al.,* 1981). In temperate climates, animals that calved before the summer have a longer interval to first ovulation than those that calved afterwards (Fonesca, *et al.,* 1983; Opsomer, *et al*., 2000). In subtropical environments, reproductive performance decrease during the warm season, as compared to cool season, where the interval to first ovulation is being longer in summer as compared to winter calving cows (Jonsson, *et al.,* 1997). Within the seasonal calving systems, PPP is shorter in dairy cows calving in late spring compared to early spring (Montgomery, 1985; Mc Dougall, *et al*., 1995). In the temperate latitudes the interval between parturition and first oestrus in dairy cows is shorter, but in winter and early spring it is longer (Montgomery, 1985). Similarly, spring calving in beef and dairy cow's causes a delay in the PP ovulation compared to autumn calving (Peters and Riley, 1982).

1.2.3.6 Nutrition

Nutrition plays a major role on the reproductive efficiency in dairy cows (Ferguson, 1996; Butler 2000; Short, *et al*., 1990). The nutrition is known to influence the metabolic and hormonal profiles, which in turn affect follicular development (Braum, *et al.,* 1986). Nevertheless, overfeeding during early PP reduces fertility (Henrichs, 1989) and excessive intake of protein during early PP delays the ovarian activity in dairy cows (Ferguson and Chaluba, 1989; Lucy, *et al.,* 1991). Malnutrition during PP suppresses LH releases and delays ovulation (Sinclair, *et al*., 2002). The physiological pathways whereby the hypothalamic-pituitary-ovarian axis is informed about the status of the animal are complex and involve several metabolites and hormones such as the growth hormone (GH), IGF-1 system, insulin and thyroid hormones (Butler and Smith, 1989). The dairy cows need energy for maintenance, milk production as well as reproduction, therefore, the EB during early PP is essential especially for resumption of ovarian activity and ovulation (Butler and Cappock 1981, Terqui, *et al.,* 1982; Villa Godoy, *et al*., 1988; Adashi, *et al.,* 1995; Beam and Butler, 1997). Cows in a negative EB have lower concentrations of thyroid hormones (Capuco, *et al.,* 2001).

1.2.3.7 Body condition score (BCS)

Body condition score (BCS) indicates the body reserves of the cow and it reflects the level of nutrition (Braun, *et al.,* 1986). The BCS has been developed to enable an accurate judgment of the body condition (Paul, 1995). The BCS at calving and 60 days after provides the most accurate prediction of PP reproductive performance (Dias and Saturina, 1992). The BCS also helps to predict the duration of PP anoestrus and conception rates (Folman, *et al.,* 1990). For each reduction in BCS, the duration of PP anoestrus was found to increase (Schallenberger, *et al.,* 1984). The cumulative percent of cows exhibiting oestrus by day 20, 40and 60 PP was higher when the cows calved at BCS of 4 (Prado, *et al.,* 1990). Conception rates were also influenced positively by BCS during early PP (Moreira, *et al.,* 2000). Cows that lost more than 0.5 units of BCS during the first 40 days PP have expressed lower conception rates at first service than cows that lost more than 0.5 BCS (Butler and Smith, 1989).

1.2.3.8 Parity

Parity is the number of times a cow had maintained a foetus beyond 260 days of gestation (Williams and Griffith, 1995; Sharma, *et al.,* 1996; Stahl, *et al*., 2006). Parity influences PPP and total number of follicular waves (Lucy, *et al.,* 1992). Dairy cows with 2nd parity have an increased fertility as compared to those at the 3rd parity dairy cows (Walters *et al.,* 2002). The emergence of good quality oocyte has been related to the number of parity (Lucy *et al.,* 1992). The 3rd parity dairy cows had higher quality oocytes near the 1st and 2nd month PP. However, the 1st and 2nd parity dairy cows had greater oocytes quality in the 3rd month PP (Lucy, *et al.,* 1991). In the 1st parity dairy cows, the interval of the first PP ovulation is reported to be longer than the 2nd and 3rd parity dairy cows (Fonesca, *et al*., 1993; Lucy, *et al*., 1992).

1.2.3.9 Age

The PPP of old cows tend to be longer as compared to heifers and middle age cows (Walters, *et al.,* 2002). The same authors reported that primiparous dairy cows have longer PPP than the multiparous. The cows reaching their seventh lactation had poor fertility (Esslemont and Kossaibati, 2000).

1.2.3.10 Uterine Involution

Involution is the reduction in the size of the genital tract which involves loss of intraluminal fluids, necrosis of the caruncular stalk, break of the superficial layers of the caruncles and formation of the lochial discharges that is expelled in copious amounts during the first 10 to 12 days PP, but expulsion is completed by day 14 to 18, if involution is normal (Odenvisk and Fredriksson, 1993). Involution of the cervix is lower than the uterine body and the decrease in the size of uterus is due to vasoconstriction which occurs at 3 to 4 minuits intervals and gradually diminishes by day 4 PP (Roberts, 1971). The uterus decreases in weight from 9 to 11 Kg at parturition to less than 1 Kg by day 25 to 30 PP (Michiel, *et al.,* 1999). Both weight and size reduction are greatest during the first few days after parturition (Toribio, *et al.,* 1995). By 20 days PP, the size of the uterus is reduced by more than 80%. By 40 days PP, the uterus is completely involuted (Michiel, *et al.,* 1999). Uterine involution affects the recovery of the hypothalamic-pituitary-ovarain axis and ovarian activity in dairy cows (Terqui, 1982). Uterine involution is more rapid in suckled beef cows than in dairy cow (Arthur, *et al*., 2001). The cows with delayed uterine involution were diagnosed with inactive ovaries (Odensvik and Fredrikson, 1993).

1.2.3.10.1 Factors influencing the uterine involution

These factors are:

- **Age**

Most of observers found that, uterine involution is more rapid in primipara than pluripara dairy cows (Arthur, *et al.,* 1998).

- **Season**

In dairy cows uterine involution occurs more quickly in the summer and spring months, but it takes longer in winter months (Michiel, *et al.,* 1999; Arthur, *et al.,* 1998).

1.2.3.11 Uterine infection

The presence of pathogenic bacteria in the uteri of the dairy cows during PP causes endomertitis, histological lesions on the endomtrium, delays uterine involution and perturbs embryo survival ((Sheldon, *et al*., 2002, Sheldon, *et al.,* 2003, Sheldon, *et al*., 2006; Foldi, *et al.,* 2006). The outcome of uterine bacterial contamination depends on the number and virulence of the organisms present as well as the condition of the uterus and its inherent defense mechanisms (Huszenicza, *et al.,* 2005). During early PP, uterine bacterial infection, bacterial products or the associated inflammation reduces FSH pituitary concentrations, suppresses LH release. Consequently, this perturbs PP ovarian follicular growth and function, which impairs ovulation in dairy cattle (Opsomer, *et al.,* 2000). The imbalance between uterine infection and the intra-uterine antimicrobial self-defense mechanisms, often results in complications, such as subclinical endometritis, puerperal metritis, clinical endometritis and false pyometra (Sheldon, *et al.,* 2002). Forty% of dairy cows were diagnosed with and treated for PP uterine infection. This PP uterine bacterial infection is associated with delayed uterine involution, appearance of the DF, and resumption of the FO, prolonged DO, lowered rate of service per conception at subsequent breeding and increased CI (Bartlett, *et al.,* 1986, Huszenicza, *et al.,* 1999). In particular, resistance of the uterus to bacterial infection and its ability to eliminate bacterial populations during early PP is influenced by ovarian activity (Foldi, *et al.,* 2006). The exact causes of uterine infection are unknown but are associated with several factors. Cows with dystocia, retained placenta, twins or stillbirths and various metabolic disorders are more likely to develop metritis than other cows (Bell and Roberts, 2007). Aberrant immune function before and after calving seems to predispose cows to sever uterine infection, which delays uterine involution. A few cows die from sever uterine bacterial infection, but dairy cows with mild uterine bacterial infection are more likely to be culled for poor reproductive performance (Fredriksson, *et al.,* 1988 Gregory, 1997). The frequent cultured pathogenic bacteria that cause PP uterine infections in dairy cows are, E. coli, Eubacterium spp, Arcanobacterium pyogenes, Pasteurella multocida, Staphylococus spp and Streptococcus spp, but some times other non-specific pathogens are found such as Campylobacter spp, Hoemophylus spp (Stevenson and Call, 1998). It has been reported that 90% of bovine uteri are infected up to day 15, 78% of bovine uteri are infected up to day 30, 50% up to day 45 and 9% up to day 60 PP (Leslie, 1983). The Fusobacterium necrophoram and Arcanobacterium pyogenes are isolated for 3 to 5 weeks PP as well as E. coli, Pasteurella spp and Proteus are isolated for 2 to 3 week PP (Konigsson, *et al.,* 2001). Dairy cows having pyogenes in their uterine fluid suffer from sever endometrits and were infertile at the first service. The duration of infertility associated with uterine bacterial infection depends on the severity and duration of inflammation (Sheldon, *et al.,* 2006., Dolezel, *et al*., 2008). Resolution of the inflammation occurs with time (Opsomer, *et al.* 2000) and the fertility is restored in the normal cow by 40 to 50 days PP.

1.2.3.12 Breed

Breed is one of the factors that influence PP reproductive efficiency in dairy cows. The influence of breed on the length of PP is very clear in dairy and beef cows. The PP in dairy breeds is shorter as compared to beef breeds and the resumption of ovarian activity PP is earlier in dairy breeds as compared to beef breeds (Savio, *et al*., 1988; Ginther, *et al*., 1989 Webb, *et al*., 1999). Most of the studies on breed effect on PP were done on European breed (Figueirede, *et al.,* 1997). Researches on PP in Zebu breed and their crosses with European breeds were sparse in the Sudan (Elshiekh and Ahmed, 2004; Ahmed and Elshiekh, 2004; Abdallah 2006). In cross-bred Sudanese dairy breeds the PPP is reported to be shorter than the local breeds (Abdallah, 2006). Reproductive physiology of Zebu breeds is not identical to that of European dairy breeds and there are many differences like DF diameter (Barros, *et al.,* 1995), ovulation moment (Pinherro, *et al.,* 1998) and oestrus length (Medrano, *et al.,* 1996). The breed also affects the conception rates (Hafez 1993). Repeat breeding is more common in high lactating dairy breeds as compared to beef breeds (Arthur, *et al.,* 1998).

1.2.3.13 Management

The most reproductive failures in dairy cattle herds are due to deficiencies in management strategies (Rhodes, *et al.,* 2003). The poor standard of herd management will lead to poor detection rates of heat (Arthur, *et al.,* 1998). Therefore, well trained herd-men are needed to detect cows on heat properly. The importance of trained herd-men was also reported by Esselmont and Kasaibati (2000) who stated that, the percentage of cows detected on heat by high qualified herd-men ranged between 82% to 97%, but percentage of cows on heat detected by unqualified herd-men was 67%. Mismanagement like over crowdness and muddy flours are known to reduce the accuracy of heat detection in dairy cows (Arthur, *et al*., 1998).

1.2.3.14 Heat stress

In heat stressed dairy cows appetite and dry matter intake are both reduced. This prolongs the PPP of negative EB, increases the CI, negatively influences the plasma concentrations of insulin, IGF-1 and glucose (Dobson and Smith, 1999). Heat stress stimulates the hypothalamic center that regulates the GnRH and LH release during follicular phase (Dobson, *et al.,* 2003). In particular, heat stress reduces the response of pituitary gland to GnRH, which consequently reduces plasma concentrations of FSH and LH (Phagat, *et al*., 1999). Similarly, heat stress during early PP affects endocrine system especially thyroid gland which releases less thyroxin under stress (Buffington, *et al.,* 1978). Heat stress is reported to alter follicular development by reducing steroid production (Wolfenson, *et al.,* 1997; Wilson, *et al.,* 1998). These effects on the developing follicles lead to damage of the developing oocyte (Rocha *et al.,* 1998). Heat stress during prepartum had residual effects on PP reproductive performance, increased 13, 14-dihydro-15- keto- PGF2α and increases the rate of uterine involution (Rutledge, *et al.,* 1999). The heat stress associates with a delayed initiation of PP ovarian activity (Putney, *et al.,* 1989; Alkatanani, *et a.,* 1999) and reduces the degree of dominance of the selected follicle (Rensis and Scaramuzzi, 2003). The heat stress is also known to reduce the growth of DF and causes incomplete dominance (Badinga, *et al.,* 1993). The dominance of the large follicle is suppressed during heat stress and the steroidogenic capacity of the theca and granulosa cells is altered (Wolfenson, *et al.,* 1997). The incomplete dominance results in ovulation of an aged follicle (Mihm, *et al.,* 1999). Heat stress that associates with hyperthermia in dairy cows could also impair oocyte quality and function (Edward and Hansen, 1997; Hyettel, *et al.,* 1997). During the heat stress the conception rate and fetal mortality increase in dairy cows (Moberg, 1984). In most cases, uterus reduces the livelihood of embryo implantation during heat stress (Dobson and Smith, 2000).

1.3 Services per conception

It is the number of services required by a female to conceive (Sastory and Thomas, 1976). There are so many factors affecting service per conception in dairy cows (Eldon, *et al.,* 1990). One of these factors is the hormonal profiles during early PP, like GnRH, LH, FSH, oestrogen and progesterone (Eldon, *et al.,* 1990), stress, season, nutrition, uterine bacterial infection, uterine involution, milk yield, mastitis, suckling, breed, parity, BCS and management have an influence on the rate of service per conception (Folman, *et al.,* 1990). The conception rate to the first service is reported to decrease during heat stress (Leva-Ocoriz, *et al.,* 1996). In the Sudan the numbers of services per conception are reported to be 1 to 5 for Butana breed (Elamin, 1969), but it is found to be 1 to 2 for imported Holstein (Ibrahim, 1983). For the cross-bred Sudanese dairy cows that were treated with GnRH during early PP was 1 to 2 numbers of services per conception (Ahmed and Elsheikh, 2004).

1.4 Days Open (DO)

Days open (DO) is the interval in days from calving to conception (Elsheikh and Ahmed, 2004; Elsheikh and Ahmed, 2005). DO is the major parameter used to assess dairy cows reproductive efficiency during PPP (Arthur, *et al.,* 1998). The DO is influenced by the same factors that influence the rate of services per conception

(Eldon, *et al*., 1990*)*. The DO was found to be 84, 131, 155 days in Hariana, Tharker and Red Sindhi dairy cows respectively (Luktuke and Bhattacharya, 1981; Vankaya, *et al.,* 1958). In the Sudan the overage DO is found to be longer (155 days) for the cross-bred Sudanese dairy cows (Elamin, *et al.,* 1981). However the DO was shorter (55 days) when the cross-bred dairy cows were treated with GnRH during early PP (Ahmed and Elsheikh, 2004).

1.5 Calving interval (CI)

It is the period between two consecutive calving (Bath, *et al*., 1985). Calving interval (CI) is influenced by the same factors that influence the rate of service per conception and the DO (Eldon, *et al*., 1990). Regular calving of dairy cows every 12 to 13 months is an important measurement of reproductive efficiency (Bath, et *al.,* 1985). The CI of Northern Sudanese Zebu cattle is found to be longer (428 days) as compared to that imported Friesian cows (361 days) raised under the tropical environment of the Sudan (Bashir, 1990; Osman and Elamin, 1990). The CI of cross-bred Sudanese dairy cows treated with PGF2α during early PP was recorded to be 359 days (Elsheikh and Ahmed, 2005).

II Repeat breeder cows

2.1 Definition:

It is defined as the cow that fails to conceive from three or more regularly spaced services with a proven fertility bull or semen without detectable reproductive abnormalities (Levine, 1999; Bage, 2003). Repeat breeder cows showed reproductive perturbations during spontaneous cyclicity, which affect oestrus and ovulation (Renee, *et al.,* 2002). If more than 15% of the cows require more than three services, repeat breeding syndrome is considered as a problem warranting further investigations (Perez, *et al.,* 2003). Repeat breeding is a multifactorial problem involving a number of extrinsic and intrinsic factors (Gustafsson and Emanuelson, 2002). These factors include bulls, malnutrition, endocrine problems, reproductive tract infection and congenital reproductive tract abnormalities such as adhesions of the ovarian bursa as well as salpingitis, cystic ovaries, endometritis and poor management (Younquist, 1988). To prevent repeat breeding, the cow should be ensured served at the correct time, insemination techniques are proper and avoid starting breeding early PP (Bage, 2003). The repeat breeder cow is the major source of economic wastage in dairy herds (Bartlett, *et al.,* 1986). The costs are increased through semen and insemination expenses, increased DO, veterinary treatments including hormonal expenses, culling and replacement costs and losses through long generation intervals.

2.2 Factors affecting repeat breeders

2.2.1 Heat detection:

Heat detection is performed by observing the daily oestrus behaviour of the cow (Hafez, 1993). The mean duration of the oestrus behaviour in dairy cows is about 10 hours (Peter and Bosu, 1988). Heat detection is more difficult to be monitored in suckling dairy cows than non sucklings (Fortune, *et al.,* 1993). Inaccurate heat detection and silent heat results in failure to present cows for services at the correct time (Perez, *et al.,* 2003). Therefore, great vigilance is needed to detect 90% of cows in heat. Many breeders miss 20 to 40% of their cows in heat and some miss more than 50% due to mis management and silent heat (Arthur, *et al.,* 1998).

2.2.2 Early embryonic death

Early embryonic death is one of the major factors that causes repeat breeding in dairy cows (Vanroose, *et al.,* 2000). The embryonic period of gestation extends from conception to the end of the differentiation stage (Garcia, *et al*., 2006). The most critical period for embryo survival after its entry into the uterine horn is reported to be 5 to 6 days after insemination (Linores, 1982; Chenault, *et al*., 1990). The main causes for early embryonic death are heat stress, uterine infection and genetic defects (Arthur, *et al.,* 1998). When the embryos die before the middle of the oestrus cycle due to the above mentioned reasons, oestrus occurs in usual interval. Therefore, cows that had embryos died before the middle of oestrus cycle are considered as repeat breeders. However, in cows whose embryos died after the middle of the oestrus cycle, the oestrus occurs in un usual interval, they are classified as non repeat breeders, since their oestrus cycle length is altered (Foote and Rick, 1999).

2.2.3 Vitamins and trace minerals

Vitamins and trace minerals are vital for sound reproductive efficiency in dairy cows (Beam and Butler, 1998). The vitamins and the trace minerals are the specific substances that are present in small amount in the diet and are essential for the body (Corah, 1996). Therefore, during ration formulation the vitamins and trace minerals should be supplemented to the feed in an ample amount that meets the requirement of the animals. Additionally, the vitamins and the trace minerals participate in wide range of the body functions. Similarly, most of them are considered as components of important enzymatic systems, which are involved in metabolic and reproductive functions (Graham, 1998). Vitamins such as A, D, E and B complex are essential for growth, maintenance and reproduction (Sprinkle, *et al.,* 2006). Repeat breeder dairy cows that receive a ration supplemented with vitamins and trace minerals had increased conception rates (Vanegas, *et al.,* 2004). The deficiency of vitamins and trace minerals are reported to be one of the major causes of repeat breeding in dairy cows (Sprinkle, *et al.,* 2006). When repeat breeder dairy cows were injected with vitamin E and Selenium before insemination their conception rates to the subsequent breeding were increased and the DO were reduced (Graham, 1998).

2.2.4 Delayed ovulation

Delayed ovulation in dairy cows is known as the emergence of the oocyte after more than 18 hours from the end of the oestrus signs (Hunter, 1994). Increment of the progesterone level above the basal concentration during oestrus cycle delays the LH surge and consequently delays ovulation (Bage, 2002). Accordingly, low conception rates are reported. The time of insemination and proper sperm transport to the site of fertilization within the functional life span of the oocyte is prerequisite for successful fertilization (Huntter, 1994). The best conception rate is observed in dairy cows that are inseminated at a synchronized ovulation than at a synchronized oestrus (Momcilovic, *et al*., 1998).

2.2.5 Non-specific uterine infection

Non-specific pathogens are mainly bacteria that enter the uterus by ascending infection and cause endometritis and/or embryonic death (Vanroose, *et al.,* 2000).The non-specific bacterial infection causes

70% or more early embryonic mortality (Christianson, 1992). Endometritis that occurs during winter leads to silent heat and as consequence in faulty insemination (Grohan, *et al.,* 1990). There is clear evidence that uterine damage in dairy cows is due to bacterial infection and 80% of infertile dairy cows had endometritis (Arthur, *et al.,* 2001). The same authors reviewed that the subclincal metritis is a major contributor to the repeat breeder syndrome of bovine subfertility. Specific and non specific infectious agents during the prepartum and PPP frequently invade the uterus that cause metritis and endometritis that lead to repeat breeding (Sheldon, *et al.,* 2006). In dairy cows, non-specific bacterial uterine infection during PP is caused mainly by Corynebacterium renale, E.coli, Pseudomonas, Staphylocous spp, Pasteurella spp and Streptococcus spp, which increase the number of services per conception and lengthen the CI (Henzen, 1999).

2.3 Postpartum (PP) intra-uterine infusion of dairy cows with diluted iodine compounds

2.3.1 Infusion of diluted Lugol's iodine

Lugol`s iodine is a transparent brown liquid of 10% potassium iodide (KI), 5% iodine (I) and 85% distilled water. It is also called Lugol`s solution. It has broad spectrum bactericidal effects, rapid on-set and short duration action. It was first developed by the French physician, Jean Lugol in 1829 (Koujan, *et al.*, 1996). The mode of action of Lugol's iodine on reproductive system is fully understood, where it causes hyperemia of the utero-mucosa and enhances uterine blood circulation, which consequently leads to high degree of iodine absorption (Sanchez, 1995). The same author reviewed that, the absorbed iodine stimulates production of thyroid hormones, which increases body's metabolism, and this increases the metabolic activity including the reproductive tract. Lugol's iodine is the cheapest and efficient drug that enhances resumption of the PP follicular waves, reduces the time taken for the appearance of the first DF, reduces the DO, CI and improves the rate of service per conception in repeat breeder dairy cows (Mutiga, 1978; Koujan, *et al*., 1996). The same above mentioned parameters were improved when PP dairy cows suffering from severe puerperal uterine infection were infused with diluted Lugol's iodine at early PP (Knutti, *et al*., 2000; Edwell, *et al*., 2004). Diluted Lugol's iodine is an effective regimen that is recommended for treatment of inactive ovaries in dairy cows (Megahed, *et al*., 1995). An early PP infusion of diluted Lugol's iodine is less expensive method than hormonal treatment, which is expensive (Megahed, *et al*., 1995).

2.3.2 Infusion of diluted Povidone iodine:

Povidone iodine is a complex of antiinfective iodine and a vecter polymer. Povidone iodine is used for the treatment of fungal, trichomonal and/or bacterial vaginitis and metritis. Diluted Povidone iodine has slow onset, long duration of action, broad spectrum antimicrobial activity, low potential to develop resistance or adverse reactions, wide availability, ease of use and with low cost (Hoang, *et al*., 2003). Iodine is gradually released after the infusion of diluted Povidone iodine (Czeizel, *et al*., 2001). In dairy cows infusion of diluted Povidone iodine during early PP is the most efficient treatment frequently used (Geiser, *et al.,* 1995). Infusion of diluted Povidone iodine during early PP in dairy cows enhances uterine involution, appearance of the first DF, initiation of the FO, reduces the

DO, CI and improves the rate of service per conception in repeat breeder dairy cows (Nakao, *et al.* 1988; Koujan, *et al.*, 1996).

CHAPTER TWO
MATERIALS AND METHODS

2.1 Study area

This study was carried out at Khartoum University dairy farm. The location of this farm is about four Km North to the Faculty of Veterinary Medicine at Shambat, North Khartoum. The average rainfall per year is 176 mm. The maximum rainfall is between July and September. Temperature is very high and it exceeds 45 °C during the days of summer months (March to June).

2.2 Animals

The study was conducted on 250 cross-bred Sudanese dairy cows (Friesian × Kenana) between 4 to 7 years old. Their BCS is between 2.5 to 3.5 according to the five-scale point system outlined by Wildman, *et al.* (1982). According to this scale, emaciated cows are scored 1, thin cows 2, average cows 3, fat cows 4 and obese cows are scored 5.

2.3 Management and feeding

Cows were milked twice a day in the morning (02:00 a.m) and evening (02:00 p.m). All animals were fed roughages ad-libitum as group, but were individually fed dairy concentration twice a day at time of milking (10kg/cow), which consist of 37% sorghum, 21%cotton seed cacke, 40% wheat brand and 2% sodium chloride. Throughout the experimental period, the cows were allowed to graze daily from 07:00 a.m to 10:00 a.m. on broad beans (Vicia faba) and Abu 70 (Sorghum bicolor) residues. The roughages were offered to the cows in the pens during the day consisted of alfa alfa (barseem) and Abu 70. After parturition; the newborn calves are separated from their dams and are fed artificially.

Table 1. Proximate analysis of supplementary feed on dry matter basis

Dry matter	NFE	CP	ASH	Moisture	Fat
92.40	52.20	20.80	4.90	7.60	6.40

2.4 Health control

Routine examination for brucellosis and vaccination against the major diseases are practiced annually. The cows are drenched with Elbendazol at a dose of 1 ml for 2.3 kg body weight (Alvenax suspension, 2.5% USP, STAR LAB. (PVT) LMD, Batch No: VPX 510. LAHORE, PAKISTAN.) to control internal parasites. Spraying of acaricides for both animals and sheds is frequently done to control external parasites.

2.5 Housing

The experimental animals are kept in open area constructed with iron bars, partially roofed with metal sheets and the flour was covered with sands. Animals are grouped according to age and level of milk production.

2.6 Uterine involution

The genital tracts of all cows were examined every other day after parturition by trans-rectal palpation till uterine involution was completed (Sheldom and Dobson, 2000, Ahmed and Elsheikh, 2004). The uterine body is described to be involuted when the size of uterine horn is equal two fingers and uterine body is palpated in the pelvic cavity (Elsheikh and Ahmed, 2004). The complete uterine endothelium involution is assumed when the ovaries are active and the animal display the first heat signs (Perez, *et al.,* 2003).

2.7 Postpartum (PP) Ovarain rebound

Postpartum (PP) ovarian rebound was assessed manually by rectal palpation every other day after parturition (Elsheikh and Ahmed, 2005). The PP ovarian activity was described to be rebound after the appearance of the first palpable DF (Perez, *et al*., 2003)

2.8 Heat detection

Oestrus detection was performed by monitoring the daily oestrus behaviour of animals, thrice a day for 20 minutes. The cow is considered to be in oestrus when it stands to be mounted by others, a clear vaginal mucous discharge hangs from her vulva and when she bellows (Hafez, 1993).

2.9 Days open (DO)

It was determined by counting the interval in days, from calving to the successful service date of those cows that conceived (Elsheikh and Ahmed, 2004; Elsheikh and Ahmed, 2005).

2.10 Cow service

Cows displayed oestrus signs in a period less than 42 days PP were not inseminated. The insemination was started after 42 days PP for the cows, which displayed oestrus cycle after that time. The cows were inseminated with a cross-bred sire with a proven fertility (Elsheikh and Ahmed, 2004; Ahmed and Elsheikh, 2004; Elsheikh and Ahmed, 2005).

2.11 Number of services per conception

It was calculated according to Elsheikh and Ahmed, (2005).It is the number of service given to the experimental animals after recrudescence of the PP oestrus and resulted in a diagnosed pregnancy not less than 42 days after service.

2.12 Pregnancy diagnosis

It was carried out for none retain cows by rectal palpation at 60 days after last cow service (Elsheikh and Ahmed, 2005).

2.13 Early PP uterine swabs collection and bacteriology

A transcevical guarded sterile disposable swabs were collected from the endometrium of each cow on day 5 PP (Noakes, *et al*., 1989 and Sheldon, *et al* 2004). The swabs were transferred to sterile test tube and were cultured within 2 hours of collection. The swabs were cultured aerobically in pre-equilibrated sheep blood agar and on MacConkey agar and incubated at 37˚C over-night. Identification of bacteria was based on the characteristic of colony, gram-stain and morphology (Barrow and Feltham, 1993). Bacterial growth on the cultured plates was scored semi –quantitatively depending on the number of bacterial colonies detected on the plate: 0: no growth; 1 < 10 colonies, 2,10 to 100 colonies, 3, 100 to 500 colonies and 4 > 500 colonies (Noakes, *et al*., 1999).

2.14 Early PP intra-uterine infusion of 1% Lugol`s or 2% Povidone iodine

One hundred and fifty milliliters of 1% Lugol's iodine (10% potassium iodide, 5% iodine, 85% and distilled water or 2% Povidone iodine (Polyvinylpyrolidiodine) were infused on day 5 after parturition in the uteri of all candidate dairy cows (Geiser, *et al.,* 1995). The apparatus used for PP uterine infusion consisted of a 2 liter stainless jug with a projected nozzle at the bottom. A rubber tube of 100 cm long connected with 30 cm catheter was fitted to the nozzle of the apparatus (Elsanousi and El tayeb, 1979).

2.15 Uterine swabs collection and bacteriology in repeat breeder dairy cows

A transcervical guarded sterile disposable swabs were collected from the endometrium of the cow that were classified as repeat breeders before intra-uterine infusion with diluted Lugol`s or diluted Povidone iodine (Noakes, *et al*., 1989). The swabs were transferred to sterile test tube and were cultured within 2 hour of collection. The swabs were cultured aerobically in pre-equilibrated sheep blood agar and on MacConkey agar. Identification of bacteria was based on the characteristic of colony, Gram-stain and morphology (Barrow and Feltham, 1993). Bacterial growth on the cultured plates was scored semi–quantitatively depending on the number of bacterial colonies detected on the plate 0 no growth; 1 < 10 colonies 2: 1 0 to 100 colonies 3: 100 to 500 colonies and 4 > 500 colonies (Noakes, *et al*., 1991).

2.16 Intra-uterine infusion of diluted Lugol`s or diluted Povidone iodine in repeat breeder dairy cows

One hundred and fifty militers of diluted Lugol`s solution (1%) or diluted Povidone iodine (2%) were infused into the uterus of the repeat breeder dairy cows at 6 hours pre-insemination or post-insemination (Geiser, *et al*., 1995). The infusion apparatus mentioned above was used.

2.2 Experimental design

2.2.1 Experiment I

This study was conducted to evaluate the influence of uterine bacterial infection during early PP on the reproductive efficiency in cross-bred Sudanese dairy cows. Furthermore, the percentage of uterine bacterial infection among PP was determined. A total of 130 dairy cows were used to examine the effects of bacterial uterine infection on six reproductive parameters. These parameters were uterine involution, appearance of the first DF, resumption of the FO, DO, rate of service per conception and CI. Endometrial swabs were collected from each cow on day 5 PP. The collected swabs were then cultured within 2 hours from collection in blood agar media and incubated at 37 C aerobically for growth. They were then cultured in MacConkey agar media for purification. The isolated bacteria were identified according to Barrow and Feltham, (1993). The uteri of 120 cows were found infected and 10 cows were uninfected. The cows that suffered bacterial uterine infection were distributed according to the level of bacterial growth (Noakes, *et al.,* 1989 Sheldon *et al.,* 2004) into 2 groups A (n = 60 cows) suffered sever uterine bacterial infection and B (n = 60 cows) suffered mild uterine bacterial uterine infection.. From the total infected cows, 40 infected cows were used to evaluate the effects of bacterial uterine infection on reproductive performance of the dairy cows. Twenty cows were severely infected (Group A) and the other 20 cows were mild infected (Group B). The six parameters mentioned above were studied to assess the PP reproductive efficiency of the selected cows.

2.2.2 Experiment II

This experiment was designed to determine the effects of intra-uterine infusion of diluted Lugol's iodine (1%) on the PP reproductive efficiency of cross-bred Sudanese dairy cows suffered bacterial uterine infection. From the remaining cows that were diagnosed as infected in experiment **I,** 40 cows suffered sever PP uterine infection were used in this experiment. The cows then divided randomly into two groups. Group (A) and group (B) 20 cow each. Group A was intra-uterine infused with 150 ml diluted Lugol's iodine (1%) on day 5 PP (Mutiga, 1978 and Edwell, *et al.* 2004). Group B was left untreated control. The parameters in question were assessed as mentioned in the materials and methods.

2.2.3 Experiment III

This experiment was designed to examine the effects of intra-uterine infusion of diluted Povidone iodine (2%) on PP reproductive efficiency in cross-bred Sudanese dairy cows suffered bacterial uterine infection. From the remaining cows that were diagnosed as infected in experiment **I,** 40 cows suffered severe PP uterine infection were used in this experiment. The cows then divided randomly into two groups. Group (A) and group (B) 20 cow each. Group A was intra-uterine infused with 150 ml diluted Povidone iodine (2%) (Nakoa, *et al.* 1988;

Geiser, *et al*., 1995 and Koujan, *et al.* 1995). Group B was employed untreated control. The parameters in question were assessed as mentioned in the materials and methods

2.2.4 Experiment IV

This experiment was conducted to study the possibility of using diluted Lugol's iodine for treating repeat breeder cow syndrome. Three parameters were studied. These parameters were DO, rate of service per conception and CI. Sixty cross-bred repeat breeder dairy cows were used in this experiment. Uterine swabs were collected from uteri of all cows. After bacterial culture, all cows were found midly infected. They were then grouped randomly into three groups. Group A (n = 20 cows) was intra-uterine infused with 1% Lugol's iodine 6 hours pre-insemination. Group B (n = 20 cows) was infused with the same solution 6 hours post-insemination. Group C (n = 20 cows) was left without treatment to serve as control according to Mutiga, (1978) and Edwell, *et al.,* (2004). The parameters in question were assessed as described in the materials and methods.

2.2.5 Experiment V

This experiment was conducted to study the possibility of using diluted Povidone iodine for treating repeat breeder cow syndrome. Three parameters were studied. These parameters were DO, rate of service per conception and CI. Sixty cross-bred repeat breeder dairy cows were used in this experiment. Uterine swabs were collected from uteri of all cows. After bacterial culture, all cows were found midly infected. They were then grouped randomly into three groups. Group A (n = 20 cows) was intra-uterine infused with 2% Povidone iodine 6 hours pre-insemination. Group B (n = 20 cows) was infused with the same solution 6 hours post-insemination. Group C (n = 20 cows) was left without treatment to serve as control (Koujan, *et al*. 1996). The parameters in question were assessed as mentioned in the materials and methods.

2.3. Statistical analysis

The results were statistically evaluated by ANOVA followed by Fisher's protect least significant difference (PLSD). Significant differences at $P<0.001$ were considered.

CHAPTER THREE

RESULTS

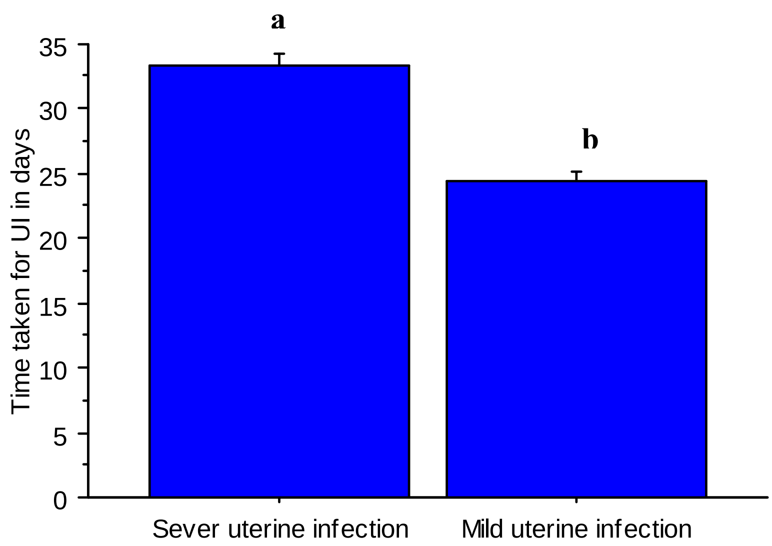
The percentage of uterine bacterial infection in cross-bred dairy cows

The uteri of 93% of the candidate dairy cows (120 cows) in this experiment were found infected during early PP. Only 7% of the employed dairy cows (10 cows) were found none infected. From the infected cows, 50% suffered sever uterine puerperal infection (60 cows). The remaining cows (60 cows) suffered mild uterine bacterial infection. The common pathogenic bacteria isolated in this study from the endometrium of the candidate dairy cows during early PP were Staphylococcus spp (36%), Streptococcus spp (31%), E coli (24%) and Pasteurella moltucida (9%).

3.1 The effects of uterine bacterial infection during early postpartum (PP) on different reproductive parameters.

3.1.1 Uterine involution

The results of this experiment showed that the time taken for uterine involution in dairy cows was significantly ($P<0.001$) influenced by uterine puerperal infection. Dairy cows suffered sever uterine puerperal infection had a prolonged time for uterine involution by about 10 days as compared to the cows suffered mild uterine puerperal infection. The mean length of the time taken for uterine involution in the dairy cows suffered severs uterine bacterial infection was 33.40 ± 0.90 days. This period is longer than that of the dairy cows (23.70 ± 0.60 days) suffered mild uterine bacterial infection (Figure 1).



Fig, 1. The influence of uterine bacterial infection during early PP on the time taken for uterine involution in dairy cows ([a,b] $P<0.001$).

3.1.2 First dominant follicle (DF)

A sever PP uterine bacterial infection in dairy cows significantly ($P<0.001$) delayed the time taken for appearance of the first DF as compared to dairy cows suffered mild PP uterine bacterial infection. The mean length of the time taken for the appearances of the first DF in the dairy cows suffered sever uterine bacterial infection was 9.60 ± 0.40 days. This value was longer than the time taken for the appearance of the first DF in dairy cows suffered mild uterine bacterial infection (7.10 ± 0.30 days) (Figure, 2).

3.1.3 Resumption of the first oestrus (FO)

As shown in figure (3) the sever PP uterine bacterial infection in the dairy cows, significantly ($P<0.001$) extended the time taken for resumption of the FO compared to the dairy cows suffered mild PP uterine bacterial infection. The mean length of the period taken for resumption of the FO in dairy cows suffered a sever PP uterine bacterial infection was 133.90 ± 4.40 days. This value was longer than that of the dairy cows with mild PP uterine bacterial infection (99.10 ± 1.60 days).

3.1.4 Days open (DO)

As showed in figure (4) the sever PP uterine bacterial infection in dairy cows, significantly ($P<0.001$) increased the DO compared to the dairy cows suffered mild PP uterine bacterial infection. The sever uterine bacterial infection prolonged the DO by 79 days. The mean length of the DO of the dairy cows with sever uterine bacterial infection was 212.40 ± 8.20 days. This value was longer than that of the dairy cows with mild uterine bacterial infection (133.80 ± 4.90 days).

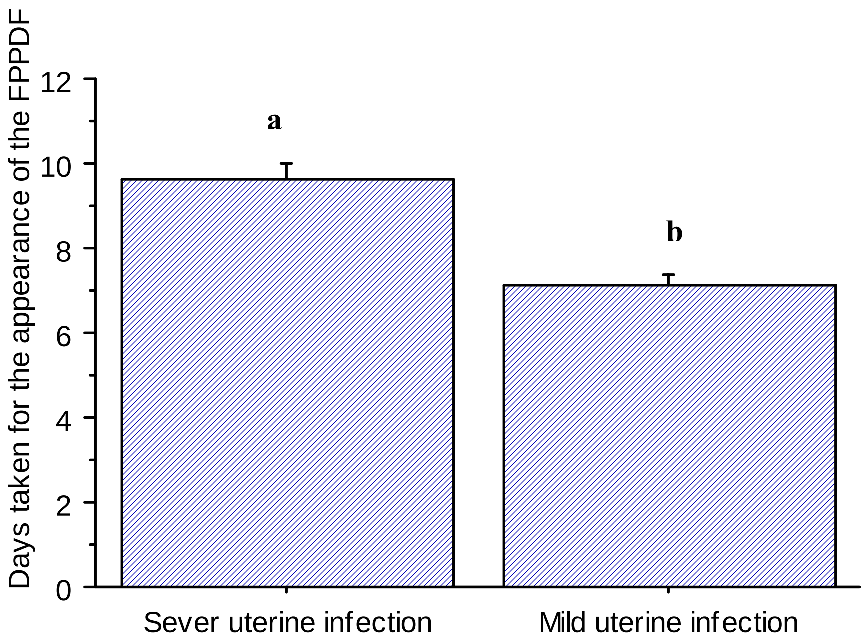


Fig.2. The influence of uterine bacterial infection on the appearance of the first DF during early PP in dairy cows ([a,b] P<0.001)

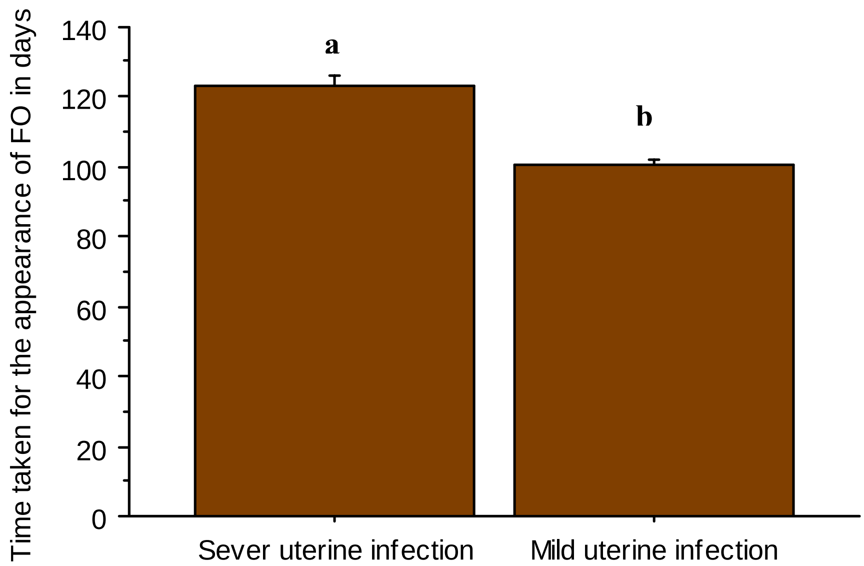


Fig. 3. The influence of uterine bacterail infection during early PP on the appearance of the FO in dairy cows ([a,b] $P<0.001$)

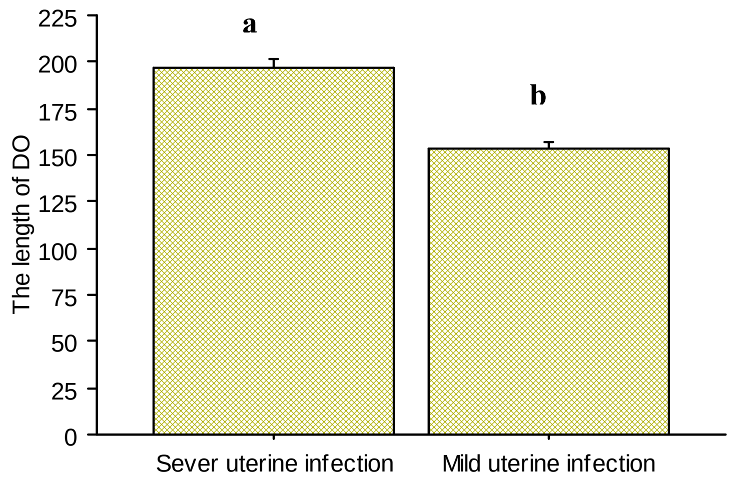


Fig.4. The influence of uterine bacterial infection during early PP on the length of the DO in dairy cows ([a,b] $P<0.001$)

3.1.5 The rate of service per conception:

As showed in figure (5) the mean rate of service per conception for the dairy cows with sever PP uterine bacterial infection was 4.50 ± 0.20 which was significantly higher (P<0.001) than that of the dairy cows with mild uterine bacterial infection (2.70 ± 0.20).

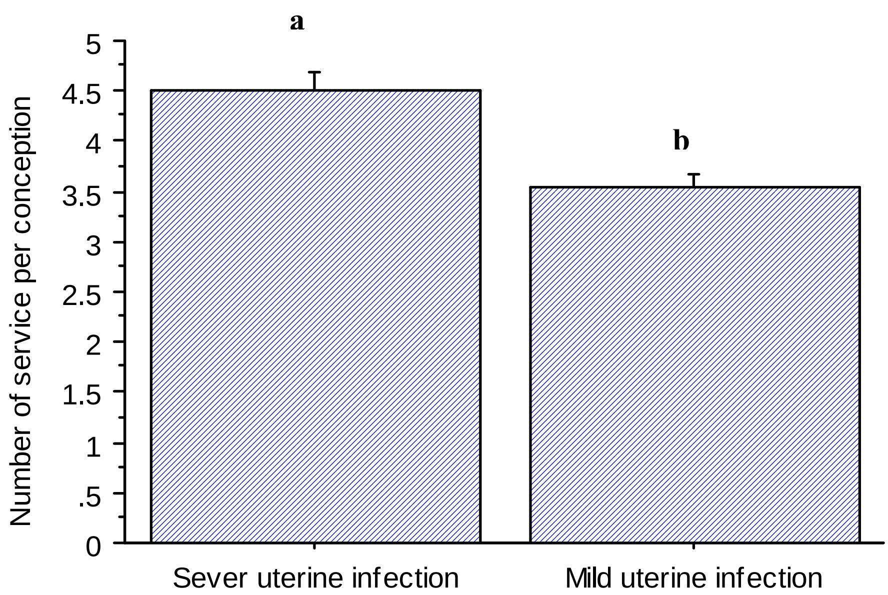


Fig.5. The influence of uterine bactrial infection during early PP on the rate of service per conception in cross-bred dairy cows ([a,b] $P<0.001$)

3.1.6 Calving interval (CI)

Figure (6) showed that the CI of the dairy cows with a sever PP uterine bacterial infection was significantly (P<0.001) longer compared to that of the dairy cows with mild PP uterine bacterial infection. The sever PP uterine bacterial infection in the dairy cows increased the CI by 75 days. The mean lengths of the CI of the dairy cows with sever PP uterine bacterial infection was 482.50 ± 9.00 days and the CI of the dairy cows with mild PP uterine bacterial infection was 407.10 ± 4.80 days.

3.2 The effects of intra-uterine infusion of diluted Lugol's iodine during early postpartum (PP) on different reproductive parameters

3.2.1 Uterine involution:

Infusion of diluted Lugol's iodine on day 5 into the uteri of PP severely infected dairy cows significantly (P<0.001) accelerated uterine involution compared to the control (Figure. 7). Infusion of diluted Lugol’s iodine during early PP accelerated uterine involution by about 16 days. The mean length of time taken for uterine involution of infused cows was 17.20 ± 0.40 days. This value was lower than that of the untreated control was (33.40 ± 0.90 days).

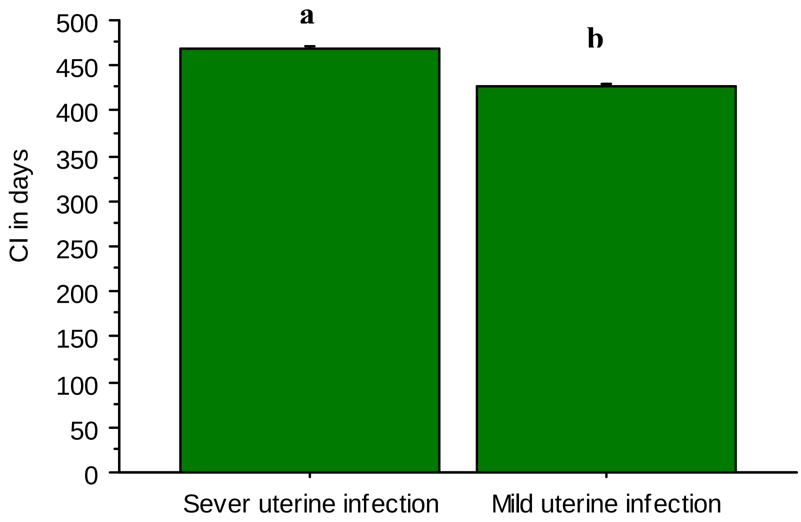


Fig.6. The influence of uterine bacterial infection during early PP on the length of the CI in dairy cows (^{a,b} $P<0.001$)

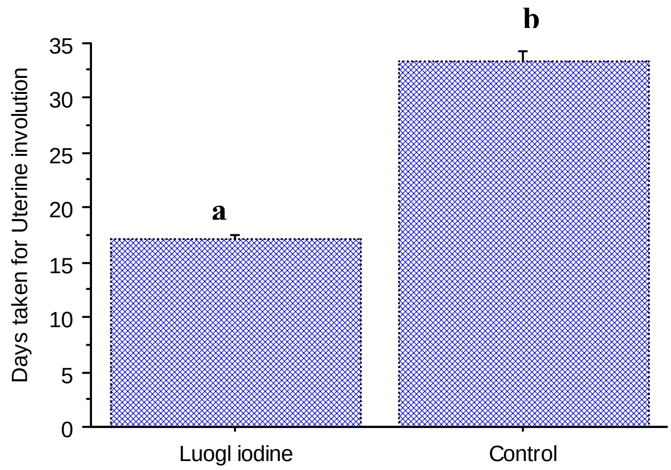


Fig. 7. The effects of intra-uterine infusion of Lugol`s iodine on day 5 PP on the time taken for uterine involution in dairy cows (^{a,b} $P<0.0001$).

3.2.2 First dominant follicle (DF)

As showed in figure (8) the time taken for appearance of the first DF for the cows that were infused with diluted Lugol’s iodine on day 5 PP was significantly reduced ($P<0.001$). The mean length of the time taken for the appearance of the first DF for these cows was 6.90 ± 0.20 days. This value is shorter than of the control (9.60 ± 0.40 days).

3.2.3 Resumption of the first oestrus (FO)

Figure (9) showed that, the dairy cows suffered sever uterine puerperal infection that were infused with diluted Lugol's iodine on day 5 PP had significantly ($P<0.001$) reduced the time taken for resumption of the FO compared to the control. The recrudescence of the FO in the treated cows was reduces by 91 days. The mean length of the time taken for resumption of the FO in treated cows was 42.70 ± 1.90 days. This value was shorter than that of the untreated control (133.90 ± 4.40 days).

3.2.4 Days open (DO)

Figure (10) showed that, the DO of the treated cows significantly ($P<0.001$) reduced compared to that of the control. The DO of the control. The DO of the control was reduced by 160 days. The mean length of the DO of the treated was 52.10 ± 2.00 days, while, that of the untreated control was 212.40 ± 8.20 days.

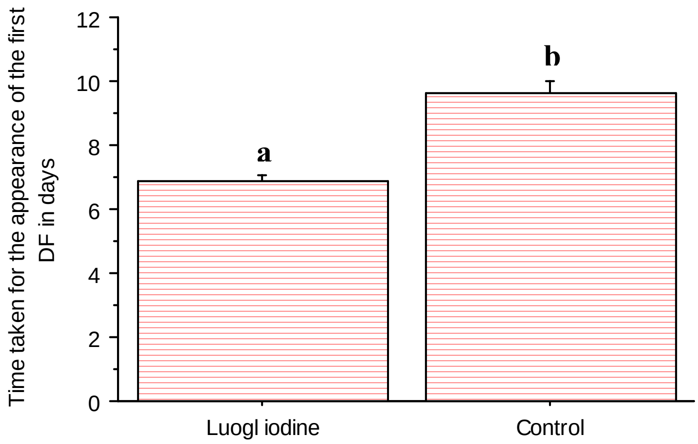


Fig. 8. The effects of intra-uterine infusion of Lugol`s iodine on day 5 PP on the appearance of the first DF in dairy cows ([a, b] P<0.0001).

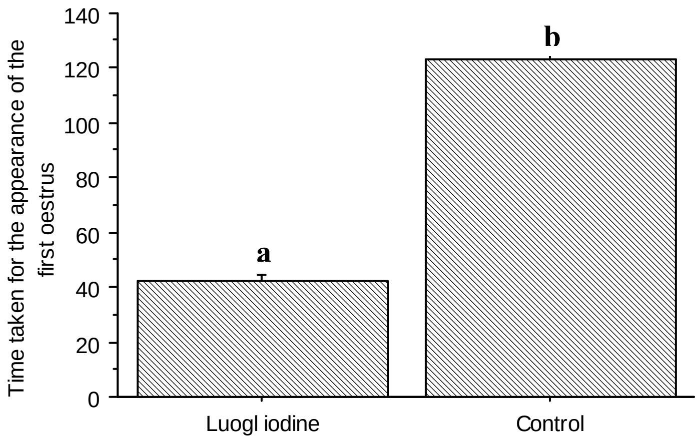


Fig. 9. The effects of intra-uterine infusion of Lugol`s iodine on day 5 PP on the appearance of the FO in dairy cows ([a,b] P<0.0001).

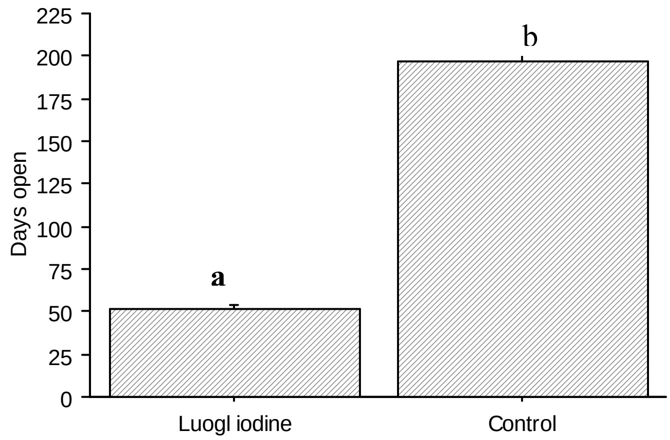


Fig. 10. The effects of intra-uterine infusion of Lugol`s iodine on day 5 PP on the length of the DO in dairy cows ([a,b] $P<0.0001$).

3.2.5 The rate of service per conception

As showed in figure (11) the rate of service per conception was improved (1.10 ± 0.10) for the treated cows. This value was significantly ($P<0.001$) lower than that of the untreated control (4.50 ± 0.20).

3.2.6 Calving interval (CI)

Figure, (12) explains that the infusion of diluted Lugol's iodine on day 5 PP significantly ($P<0.001$) reduced the CI. Infusion with diluted Lugol's iodine on day 5 PP shortened the CI by about 158 days. The mean length of the CI reported for the treated cows was 324.90 ± 6.50 days, while the CI of the untreated control was (482.50 ± 9.00 days).

3.3 The effects of intra-uterine infusion of diluted Povidone iodine during early postpartum (PP) on different reproductive parameters

3.3.1 Uterine involution

As showed in figure (13), involution of the uterus was significantly ($P<0.001$) accelerated by infusion of diluted Povidone iodine on day 5 PP in the severely infected dairy cows. Infusion of diluted Povidone iodine during early PP accelerated uterine involution by about 20 days. The mean length of the time taken for uterine involution of the treated cows was 13.10 ± 0.30 days. Contrarily, the involution of the uterus of the control was (33.40 ± 0.90 days).

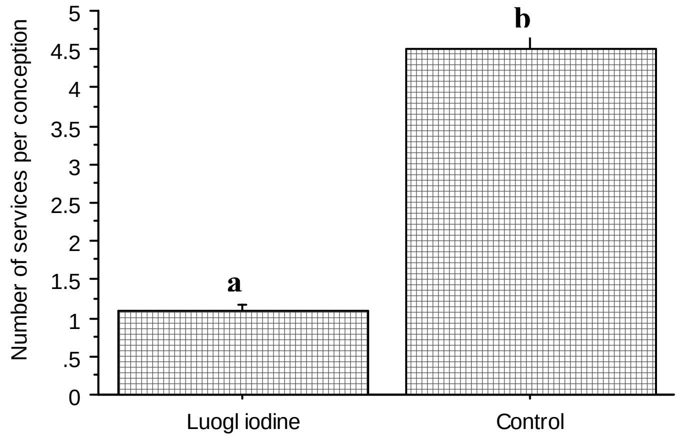


Fig. 11. The effects of intra-uterine infusion of Lugol`s iodine on day 5 PP on the rate of service per conception in dairy cows ([a, b] P<0.0001).

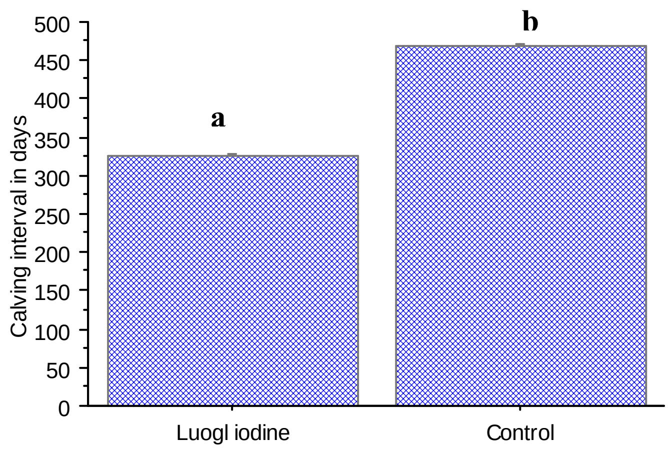


Fig.12. The effects of intra-uterine infusion of Lugol`s iodine on day 5 PP on the length of the CI in dairy cows ([a,b] P<0.0001).

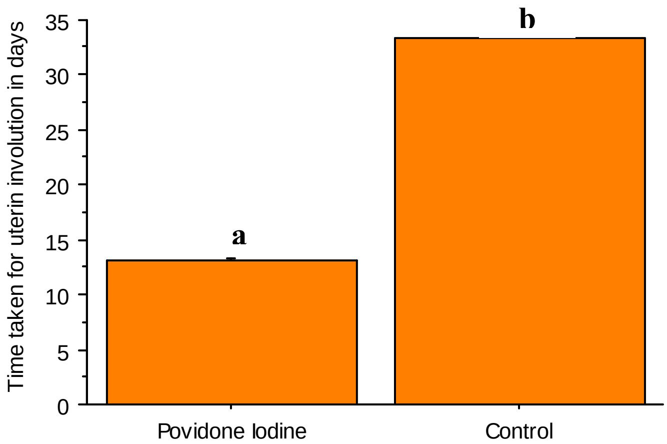


Fig.13. The effects of intra-uterine infusion of Povidone iodine on day 5 PP on the time taken for uterine involution in dairy cows ([a,b] $P<0.0001$).

3.3.2 First dominant follicle (DF)

As showed in figure (14) the time taken for appearance of the first DF for the cows that were infused with diluted Povidone iodine on day 5 PP was significantly reduced (P<0.001). The mean length of the time taken for the appearance of the first DF for these cows was (6.75 ± 0.30 days). This value was shorter than of the control (9.60 ± 0.40days).

3.3.3 Resumption of the first oestrus (FO)

As shown in figure. (15) Infusion of diluted Povidone iodine into the uteri of severely infected PP dairy cows significantly reduced (P<0.001) the duration of the resumption of the FO. Infusion with diluted Povidone iodine minimized the time taken for the resumption of the FO by about 85 days. The mean length of the duration of the FO for the treated cows was 48.80±1.20 days, while that of the control was (133.90 ± 4.40 days).

3.3.4 Days open (DO)

As showed in figure (16) the severely puerperal infected dairy cows that were infused with diluted Povidone iodine had a significantly (P<0.001) short DO compared to the control. Infusion of diluted Povidone iodine on day 5 PP minimized the time taken for the resumption of the DO by about 155 days. The mean length of the DO of the treated cows was 57.20 ± 2.60 days. This value was shorter than that of the control (212.40 ± 8.20 days).

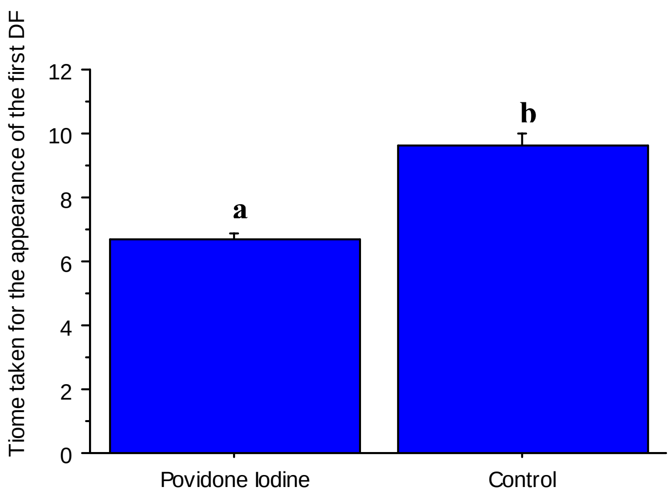


Fig.14. The effects of intra-uterine infusion of Povidone iodine on day 5 PP on the appearance of the first DF in dairy cows ([a,b] P<0.0001).

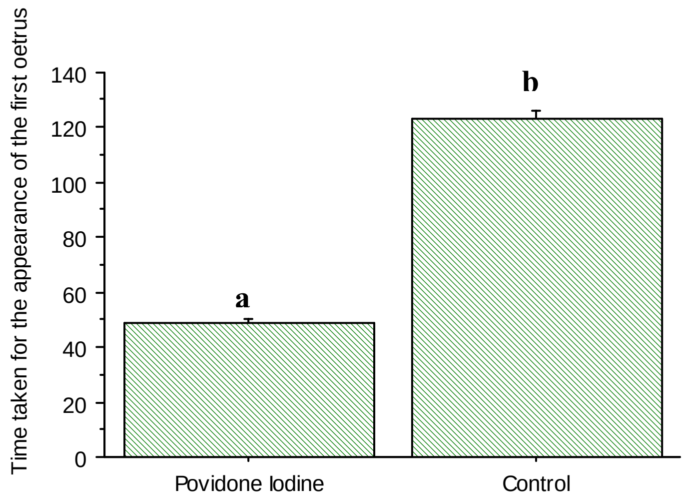


Fig.15. The effects of intra-uterine infusion of Povidone iodine on day 5 PP on the appearance of the FO in dairy cows ([a,b] $P<0.0001$).

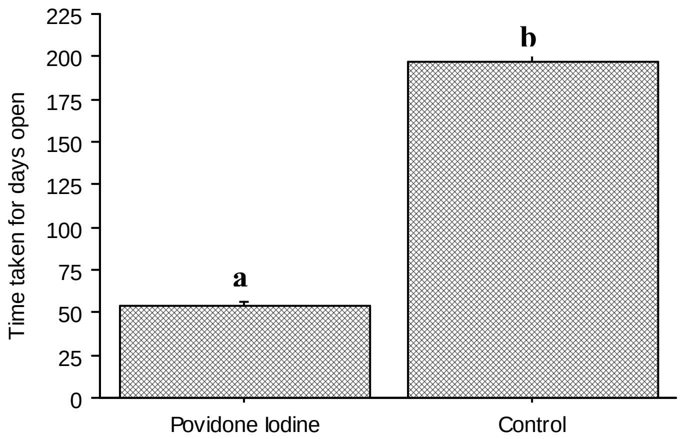


Fig.16. The effects of intra-uterine infusion of Povidone iodine on day 5 PP on the length of the DO in dairy cows (^{a,b} $P<0.0001$).

3.3.5 The rate of service per conception

Figure (17) explains that, the rate of service per conception for the severely infected dairy cows that were infused with diluted Povidone iodine on day 5 PP was significantly ($P<0.001$) reduced (1.40 ± 0.10) compared to that of control (4.50 ± 0.20).

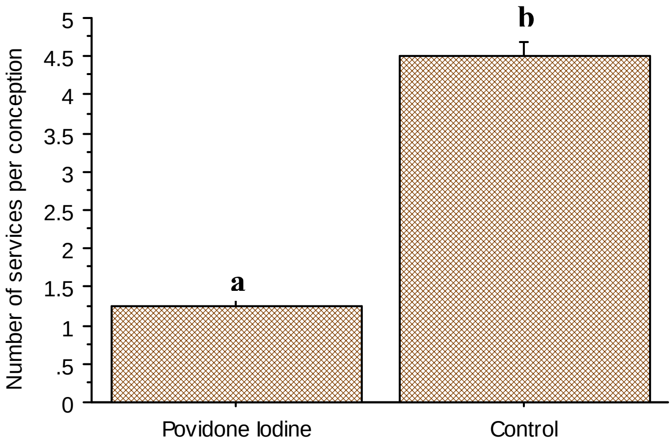


Fig.17. The effects of intra-uterine infusion of Povidone iodine on day 5 PP on the rate of service per conception in dairy cows ([a,b] $P<0.0001$).

3.3.6 Calving interval (CI)

Figure (18) explains that, the severely infected dairy cows that were infused with diluted Povidone iodine on day 5 PP had a shorter ($P<0.001$) CI as compared to the control. Infusion of diluted Povidone iodine on day 5 PP minimized the CI by about 151 days. The mean length of the CI of the treated cows was 331.70 ± 2.9 days and the CI of the control was 482.50 ± 9.00 days.

3.4 The effect of infusion of diluted Lugol's iodine pre or post insemination on some reproductive parameters in repeat breeder dairy cow

All the candidate cows (120 cows) in experiment four and five suffered endometritis. The main pathogens isolated from the uteri of the repeat breeder dairy cows were Staphylococus spp (27%), Streptococus spp (25%) , E. coli (38%) and Pasteurella moltucida (10%).

3.4.1 Days open (DO)

As shown in figure (19) the DO of the repeat breeder dairy cows was significantly ($P<0.001$) reduced by infusion of Lugol's iodine pre/post insemination compared to the control. The mean length of the DO for the treated repeat breeder cows was 135.50 ± 4.20 days for pre insemination treated cows and 146.50 ± 6.20 days for post insemination treated cows. While the DO of the control was 218.90 ± 6.30 days.

3.4.2 The rate of service per conception

Figure (20) showed that, the rate of service per conception in repeat breeder dairy cows was significantly ($P<0.001$) improved when diluted Lugol’s iodine was infused pre/post insemination (1.00 ± 0.00 and 1.40 ± 0.10 respectively). Hundred percent of the candidate cows that were infused with diluted Lugol's iodine pre-insemination conceive from the first insemination, but only 60% of the repeat breeder dairy cows that were infused post-insemination conceived from the first insemination compared to the control where most of the cows conceived after more than three inseminations (4.80 ± 0.20).

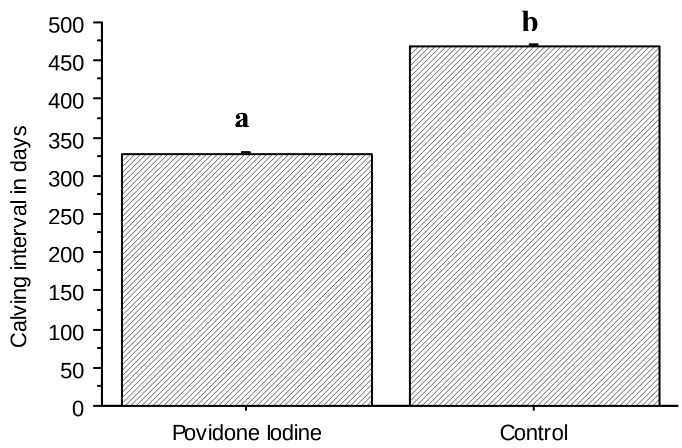


Fig.18. The effects of intra-uterine infusion of Povidone iodine on day 5 PP on the length of the CI in dairy cows ([a,b] P<0.0001).

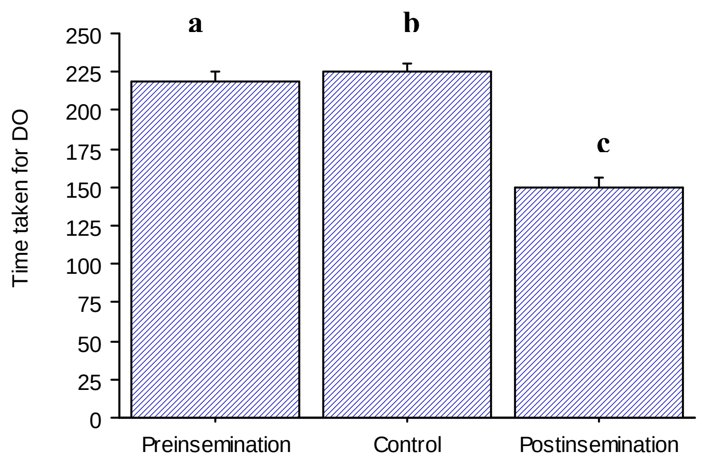


Fig.19 The effects of intra-uterine infusion of diluted Lugol`s iodine pre/post insemination on the length of the DO in repeat breeder dairy cows ([a,b,c] $P<0.001$).

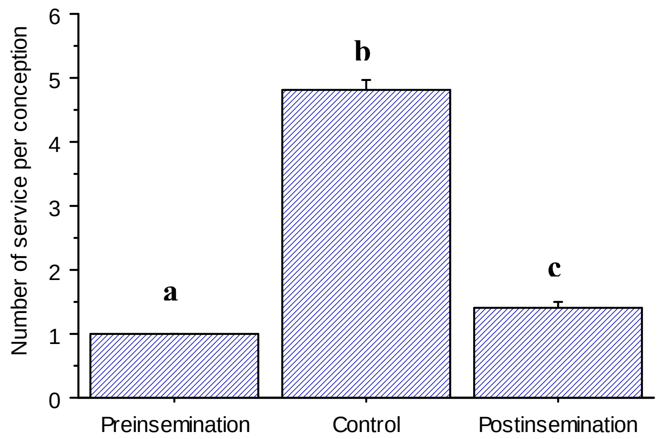


Fig. 20 The effects of intra-uterine ifusion of Lugol's iodine pre/post insemination on the rate of service per coception in repeat breeder dairy cows ([a,b, c] P<0.001).

3.4.3 Calving interval (CI)

Figure (21) explained that the repeat breeder dairy cows that were infused with diluted Lugol's iodine at pre/post insemination had a shorter CI as compared to the control ($P<0.001$). Infusion of diluted Lugol's iodine at pre/post insemination in repeat breeder dairy cows shortened the CI by 67 and 56 days respectively. The mean lengths of the CI of the repeat breeder dairy cows that were infused with diluted Lugol's iodine at pre/post insemination were 409.90 ± 4.30 and 420.30 ± 6.50 days respectively, while, the CI of the control was (476.80 ± 7.50 days).

3.5 The effect of infusion of diluted Povidone iodine pre or post insemination on some reproductive parameters in repeat breeder dairy cows

3.5.1 Days open (DO)

As shown in figure (22) repeat breeder dairy cows that were infused pre/post insemination with Povidone iodine, the DO were significantly ($P<0.001$) reduced compared to the control. The mean length of the DO for the repeat breeder dairy cows that were infused with 2% Povidone iodine pre/post insemination were 134.30 ± 5.70 days and 151.40 ± 7.10 days respectively. While the DO of the control was 227.70 ± 6.40 days.

3.5.2 The rate of service per conception

Figure (23) showed that, in repeat breeder dairy cows the rate of service per conception was significantly ($P<0.001$) improved by infusion of diluted Povidone iodine pre/post insemination (1.30 ± 0.10 and 1.90 ± 0.20 respectively). Seventy percent of the candidate cows that were infused with diluted Povidone iodine pre-insemination conceive from the first insemination, but only 25% of the repeat breeder dairy cows that were infused post-insemination conceived from the first insemination as compared to the control where most of the cows conceived after more than three inseminations (5.10 ± 0.20).

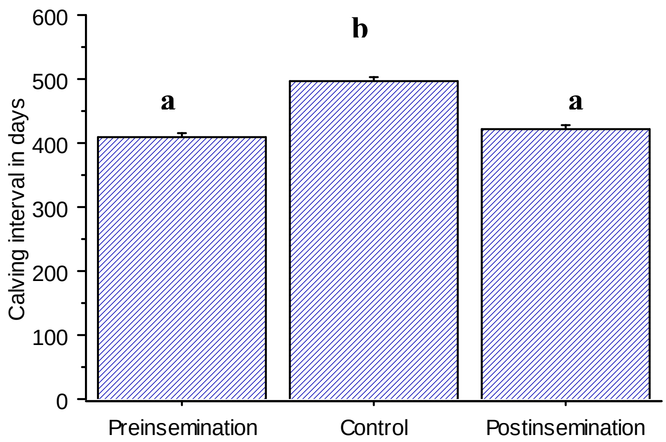


Fig.21 The effects of intra-uterine infusion of Lugol's iodine pre/post insemination on the length of the CI of repeat breeder dairy cows ([a,b] $P<0.001$).

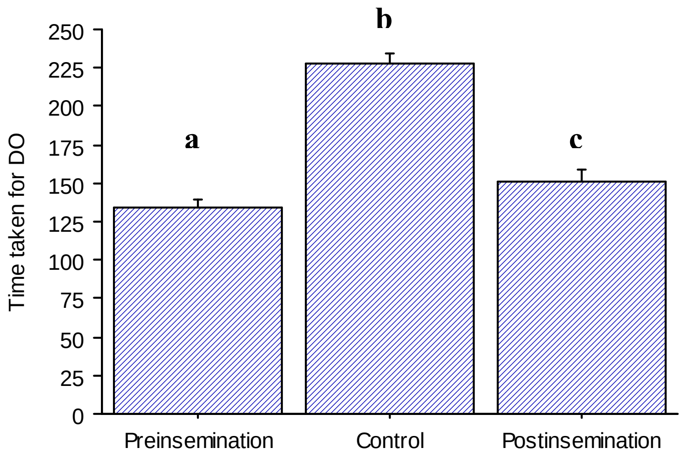


Fig.22. The effects of intra-uterine infusion of diluted Povidone iodine pre/post insemination on the length of the DO in repeat breeder dairy cows ([a,b,c] $P<0.001$).

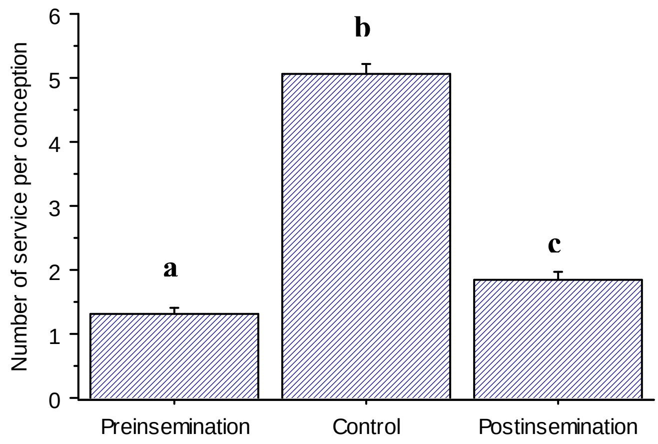


Fig.23. The effects of intra-uterine infusion of diluted Povidone iodine pre/post insemination on the rate of service per conception in repeat breeder dairy cows ([a,b,c] $P<0.001$).

3.5.3 Calving interval (CI)

Figure (24) indicates that, the repeat breeder dairy cows that were infused with diluted Povidine iodine pre/post insemination had a shorter CI as compared to the control ($P<0.001$). Pre/post insemination, infusion of diluted Povidone iodine in repeat breeder dairy cows decreased the CI by 92 and 75 days respectively. The mean length of the CI of the repeat breeder dairy cows that were infused with diluted Povidone iodine at pre/post insemination were 407.90 ± 5.80 and 424.70 ± 7.50 day respectively and the CI of the control was 499.90 ± 5.90 days.

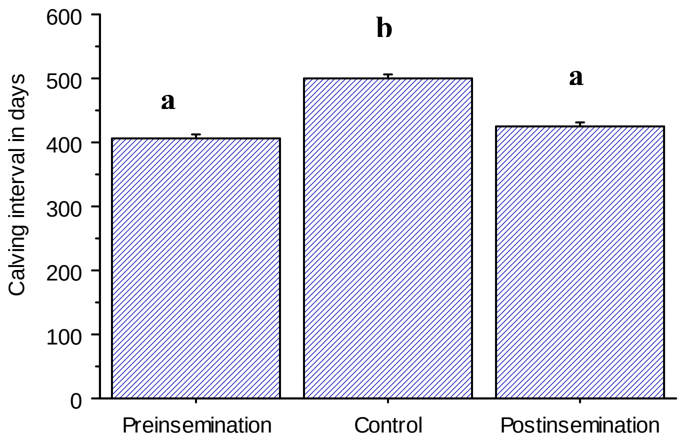


Fig.24. The effects of intra-uterine infusion of diluted Povidone iodine pre/post insemination on the length of CI in repeat breeder dairy cows ([a,b] $P<0.001$).

CHAPTER FOUR
DISCUSSION

This study confirms that, the reduced PP reproductive efficiency and the repeat breeder cow syndrome in the Sudan are mainly due to uterine bacterial infection. Furthermore, this study indicated that, these problems could be solved by intra-uterine infusion of iodine compounds such as Lugol's and Povidone iodine.

Forty percent of dairy cows were diagnosed with and treated for PP uterine bacterial infection (Leslie, 1983). In particular 90% of bovine uteri are infected up to day 15, 78% up to day 30, 50% up to day 45 and 9% up to day 60 PP (Sheldon, *et al*., 2002; Sheldon, *et al.,* 2003, Sheldon, *et al*, 2006; Foldi, *et al.,* 2006). This PP uterine bacterial infection delays uterine involution, initiation of the first follicular wave, appearance of the first DF and the occurrence of the FO. Consequently, it prolongs the DO, increases the rate of service per conception at subsequent breeding and increases the CI (Bartlett, *et al.,* 1986, Huszenicza, *et al.,* 1999). The above findings agree with our findings in which 92% of uteri of the PP dairy cows were found infected. The major pathogenic bacteria isolated from uteri of the PP dairy cows were Staphellococus spp, Streptococus spp, Pasteurella moltucida and E. Coli. The uterine bacterial infection during early PP in the dairy cows is presumply due to poor sanitation and hygiene during normal delivery, assisted per vaginum delivery or manipulation of the retained foetal membranes. In addition, contamination of the genital tract of the cows could be via the descending bacterial infection. Uterine bacterial infection, bacterial products or the associated inflammation reduce pituitary FSH, suppress LH release and perturb PP ovarian follicular growth and function, which delays the ovulation in dairy cattle (Opsomer, *et al.,* 2000). The pathogens found in this study are similar to the pathogens reported in several studies that were reviewed elsewhere (Stevenson and Call, 1998). The presence of these pathogenic bacteria in the uterus during this critical period cause endomertitis and induces histological lesions on the endometriam. It also delays the uterine involution, initiation of follicular waves, the recrudescence of the FO and it perturbs embryo implantatation. The effect of uterine bacterial contamination during early PP depends on the number and virulence of the organisms present as well as the condition of the uterus and its inherent defense mechanisms (Huszenicza, *et al.,* 2005). Dairy cows with mild uterine bacterial infection during early PP suffered less than the dairy cows with sever PP uterine bacterial infection. Ninety two percent of the cows in this study had bacterial infection during early PP. The cows that suffered severe PP uterine bacterial infection had a longer time taken for uterine involution than the dairy cows that suffered from mild PP uterine bacterial infection. This can be attributed to the sever endometritis that occurred during early PPP which led to the damage of the endomerium and suppressed $PGF_{2\alpha}$ which is essential in this critical period for uterine involution (Elsheikh and Ahmed, 2004). This result is in consistent with the result of Sheldon (*et al*., 2006) and Dolezel (*et al*., 2008) who reported that uterine bacterial infection during early PP delays uterine involution. This study also confirms that, the initiation of the first follicular wave and the appearance of the first DF were affected by uterine bacterial infection during early PP. The dairy cows suffered severe PP uterine bacterial infection had a prolonged period for the initiation of the first follicular wave and the appearance of the first DF. These findings match with the findings of Huszenicza *et al* (1999) who emphasized that dairy cows that suffered

PP uterine bacterial infection had a prolonged time for initiation of the first follicular wave and the appearance of the first DF. The findings of this study also indicated that dairy cows which suffered severe uterine bacterial infection during early PP had a prolonged period for the recrudescence of the FO. This could be attributed to the delayed uterine involution and the reduced pituitary FSH and LH surge (Dobson, *et al*., 2000). This finding agrees with the finding of Opsomer *et al*., (2000) who reported that dairy cows that suffered uterine bacterial infection during the first week PP had a delayed recrudescence of the FO. Consequently, the DO was extended, the rate of service per conception at subsequent breeding was increased and the CI was longer. These findings also match with the findings of several studies that recorded the effect of uterine bacterial infection during early PPP on the DO, CI and the conception rate (Bartlett, *et al*., 1986; Huzenicza, *et al*., 1999).

The uteri of 92% of the PP cows used in this research were found infected during early PP. Thus, they experienced a dramatic decrease in PP reproductive efficiency. The results of this study demonstrated that, intra-uterine infusion of 1% Lugol's iodine during the fist week PP accelerated uterine involution of the treated cows. Lugol's iodine is known to increase hyperemia of the endometrium, increases its blood supply and the iodine in Lugol’s solution when its absorbed transferred to the thyroid gland and associated with thyroid binding globulin (TBG) in the thyroid gland to produce thyroxin under control of hypothalamus (TRH) and pituitary gland (TSH) which is essential for internal cellular metabolism, improvement of follicular development, ovulation and maintenance of pregnancy (Yu and Tak-yin, 1993). Furthermore, Lugol’s iodine has a bactericidal effect (Sheldon, *et al*., 2006). These activities of iodine will enhance the healing of the endometrium and restorement of its activities to produce $PGF_{2\alpha,}$ which is responsible for the accelerated uterine involution of the dairy cows. The internal cellular metabolism of the reproductive system including the ovaries will also be improved by infusion of diluted Lugol's iodine (Sarkar, 2006). Thus, in this study the initiation of the first follicular wave, the appearance of the first DF and the recrudescence of the FO were accelerated. The DO and CI were reduced. Moreover, the rate of service per conception at subsequent breeding was improved. These results are in agreement with those of Knutti *et al*., (2000) and Edwell *et al*., (2004) who reported that intra-uterine infusion of diluted Lugol's iodine during early PP reduces the time taken for uterine involution, resumption of the first follicular wave and appearance of the first DF, recrudescence of the FO, minimizes DO, CI and improves the rate of service per conception in severely uterine bacterial infected dairy cows. This increased conception rate is probably due to the improvement of the uterine environment, as a result of the potent bactericidal effect of the solution. When the uterine environment is improved the ovary will be able to produce high quality oocyte and the uterus will be capable to produce more uterine milk which helps embryo implantation (Oakley, 1992). Similarly, intra-uterine infusion of 2% Povidone iodine into the uteri of severely infected dairy cows during early PP improved the reproductive efficiency of the candidate dairy cows. This result agrees with the result of Nakao *et al* (1999) who reported that, infusion of diluted Povidone iodine into the uteri of infected dairy cows during the early PP improves the reproductive performance of the dairy cows. This improvement is probably due to the gradual release of iodine that transferred to the thyroid gland and associated with thyroxin binding globulin (TBG) to release enough thyroxin hormone which triggered the hypothalamic-pituitary axis, the release of high levels of GnRH that initiated production of FSH and LH, emergence of high quality oocyte, release of uterine milk that increase the life-hood of the embryo implantation (Sarkar, 2006) and the potent bactericidal effects of Povidone iodine (Oakley, 1992).

The main pathogenic bacteria that entered the uterus by and ascending or descending infection isolated from the genital tracts of the repeat breeder dairy cows and caused endometritis in this study were, Staphylococus, Streptococus, E. coli and Pasteurella moltucida. The repeat breeder dairy cows were found to have increased rate of service per conception, a long DO and a long CI. This is probably due to clinical or subclinical endometrits that altered the pH of the genital tract of the infected cows and consequently led to failure of conception. This result is in consistent with the result of Grohan and Saoloemi (1990) who reported that endometritis leads to silent heat, failure of inseminations and repeat breeding in dairy cows.

The infusion of diluted Lugol's iodine into the uteri of the repeat breeder dairy cows pre-insemination had improved conception rate. This improvement could be due to the potent bactericidal effects of the solution, adjustment of the reproductive tract tissues pH before insemination, slight irritation of the endometriam which enhanced uterine blood circulation, increased defense mechanisms of the reproductive system that become reasonably efficient (Sarkar, 2006). Consequently, the DO and CI were reduced in the repeat breeder dairy cows. However, the infusion of diluted Lugol's iodine into the uteri of the repeat breeder dairy cows post-insemination had less effects on the rate of service per conception. This difference definitely is due to sub-clinical or clinical endomeritis that occurred in the genital tract of the repeat breeder dairy cows, which disturbs the uterine pH and decreases the livelyhood of the embryo growth (Edwell, *et al*., 2004). This result is in consistent with that of Koujan, *et al* (1996) who reported that infusion of diluted Lugol's iodine post-insemination in repeat breeder dairy cows reduced the rate of service per conception.

Similarly, in repeat breeder dairy cows the rate of service per conception was improved when the cows were infused pre-insemination with diluted Povidone iodine. This improvement is surely due to potent bactericidal effects of diluted Povidone iodine, which clean up the bacterial populations from the reproductive tract, enhanced uterine blood circulation, gradual release, and absorption of iodine that stimulate the thyroid gland to release thyroxin which improve follicular development, ovulation, maintenance of pregnancy, intrinsic factors and maintenances of the reproductive tract tissues pH (Gotherdi, 1991). However, infusion of diluted Povidone iodine post-insemination had slightly reduced effect on the rate of service per conception. This difference probably was due to sub-clinical or clinical endomeritis that occurred in the genital tract of the repeat breeder dairy cow, which disturbs the uterine pH that decreased the livelyhood of the embryo growth before implantation. This result matches with the result of Nakao *et al* (1999) who mentioned that repeat breeder dairy cows that were infused with diluted Povidone iodine post-insemination gave a little bit improvement on conception rate.

The iodine is essential for initiation of follicular wave dynamic, ovarian activities and improvement of rate of service per conception in dairy cows (Bailey, *et al.,* 1999; Sarkar, 2006). Iodine deficiency is mainly exaggerated by heat stress that is common in the Sudan. The reduced iodine in the body of the dairy cows will lead to reduction in thyroxin release that is essential for herd fertility (Sarkar, 2006). Thus, from the results of this study it is clear that the use of iodine compounds improved the reproductive efficiency PP and repeat breeder dairy cows.

CONCLUSIONS

From this study, it is concluded that, the reduced fertility PP and repeat breeder cows is likely to be due to subclinical or clinical endometritis. This infertility can be managed by infusion of 1% Lugol's or 2% Povidone iodine.

The following conclusions could be drawn:-

1- The uterine involution in puerperal infected dairy cows will not take place before 33 days.

2- The follicular wave and the emergence of the first DF will not occur before day 9 PP in severely infected dairy cows.

3- An observable FO will not be recorded before day 133 PP in severely infected dairy cows.

4- The DO and CI of severely infected dairy cows will not be less than 212 and 482 days respectively.

5- The cows having infected uteri need a high rate of service per conception.

6-Infusion of diluted Lugol's iodine during early PP in the dairy cows reduces the time taken for uterine involution by 16 days, the time taken for recrudescence of the FO by 91 days, DO by 160 days and CI by 158 days.

7- Infusion of diluted Povidone iodine during PP in the dairy cows decreases the time taken for uterine involution by 20 days, the time taken for recrudescence of the FO by 85 days, DO by 155 days and CI by 151 days.

8- Infusion of diluted Lugol's iodine pre-insemination in repeat breeder dairy cows shortens the DO by 83 days, while its infusion post-insemination reduces the DO by 72 days. Consequently, the CI is reduced.

9- Infusion of diluted Povidone iodine pre-insemination in repeat breeder dairy cows shortens the DO by 93 days, while its infusion post-insemination reduces the DO by 76 days. Consequently, the CI is reduced.

RECOMMENDATIONS

This research has been conducted to improve the reproductive performance of the dairy cows via intra-uterine infusion of diluted Lugol's or diluted Povidone iodine during early PP which has the potential of being economically justifiable. Day open (DO), conception rate and CI in repeat breeder dairy cows have been successfully reduced by this protocol. Lugol's iodine 1% and Povidone iodine 2% are good example of compromise between functionality and cheap price. In addition, they are easy to obtain, easy to made, easy to store, easy to transport and profitable treatment that can be applied routinely to improve the fertility of the dairy cows. Accordingly, the following recommendations are suggested:-

1. Sanitation should be adopted as much as possible, start with clean hands and use disposable equipment especially during assisted per vaginum delivery, caesarian sections or manipulation of the retained faetal membranes are the best defense to avoid bacterial contaminations of the genital tract of the candidate cows which is known to reduce the PP reproductive performance.
2. In dairy farms, there should be a calving room of especial design to secure relatively clean delivery and minimize the risk of PP uterine bacterial infection, which is known to reduce the reproductive performance of the dairy cows.
3. Routine dipping of the teats of the udder of the late pregnant dry lactating cows with diluted Lugol’s or diluted Povidone iodine, which are known for their potent bactericidal activities, should be done to avoid occurrence of clinical or subclinical mastitis as early as PP to avoid ascending endometritis.

4. Infusion of diluted Lugol's iodine or diluted Povidone iodine during early PP is highly recommended to improve the PP reproductive efficiency of the dairy cows.
5. It is much better to infuse diluted Lugol’s or diluted Povidone iodine pre-insemination in repeat breeder dairy cows than their infusion post-insemination.
6. Pre-insemination infusion of diluted Lugol's iodine seems to be one of the excellent choices, for treatment of repeat breeding syndrome in the dairy cows.
7. Infusion of iodine compounds is highly recommended for improvement of conception rate in dairy cows especially in the tropical zones where the dairy cows suffer from heat stress, which is known to reduce thyroxin hormone, and consequently a reduced conception rate.
8. Dairy cows that suffer from subclinical or clinical endometritis as early as PP should be infused with diluted Lugol's or diluted Povidone iodine on day 5 PP to improve the reproductive efficiency during this critical period.
9. Infusion of diluted iodine compounds help to avoid antibiotic and hormonal residues in both milk and meat that they become save enough for human consumption.
10. Infusion of diluted Lugol's iodine is an effective regimen that is recommended for treatment of inactive ovaries since it is at the same time caused by iodine deficiency.

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Appendix I. Different reproductive parameters of PP dairy cows examined for uterine bacterial infection in cross-bred dairy cows

Exp No: 1 A: Sever PP uterine bacterial infection

Serial No	UI	First DF	FO	RSC	DO	CI	Remarks
1	35	8	150	4	213	483	++++ Staph spp Strepspp,E coli
2	30	7	120	5	204	477	+++ + Stah spp, StrteptSp
3	26	9	110	5	194	470	++++ Staph spp, strept spp
4	36	7	112	4	175	450	++++ Strep Spp, E coli
5	30	10	120	4	183	455	++++ Strep Spp, Staph spp. E coli
6	36	8	125	3	167	441	++++ Strep Spp, E coli
7	32	11	118	6	223	493	++++ Strep Spp, Staph, E coli
8	30	9	115	3	157	444	++++ Srep Sp, Staph spp , E coli
9	28	11	112	5	196	467	+++ +Srep Spp, Stapg, E coli
10	36	9	125	5	209	416	+++ Staph Spp, Strep Spp, E coli
11	40	14	120	4	183	455	++++ Staph Spp, Strep Spp, E coli
12	36	10	123	4	186	460	++++ Staphspp, Strept sppE coli
13	35	11	120	5	204	474	+++ Srep Spp, Staph Spp
14	30	9	128	6	233	506	++++ Strep Spp Staph Spp, Paturellaspp
15	36	8	118	4	181	458	++++ Srep Spp, Staph Spp
16	30	12	115	5	199	479	++++ Step Spp, Staph Spp, E coli
17	36	10	125	4	188	463	++++ Staph Spp, E coli
18	30	9	142	5	226	498	++++ Staph Spp, E coli
19	36	12	148	4	211	481	++++ Staph Spp, Strep Spp
20	40	8	122	5	206	480	++++ Strep Spp , Pasturella Spp

B: Mild PP uterine bacterial infection in cross-bred dairy cows.

Serial	UI	First DF	FO	RSC	DO	CI	Remarks
1	21	6	110	4	173	446	++ Strep Spp, Staph Spp
2	25	7	95	3	137	407	++ Staph Spp, E coli
3	30	8	102	4	165	437	+ +Staph Spp, Pasturellaspp
4	21	5	107	3	149	425	+ Staph Spp
5	28	8	96	3	138	410	++ Strep Spp, E coli
6	21	7	100	4	163	433	+ Strep Spp. Staph Spp
7	25	7	98	4	161	436	++ E coli, Strep spp
8	28	8	96	3	132	404	+ Strep Spp, E coli
9	26	8	112	3	152	429	++ Strerp Spp, E coli
10	21	6	95	4	158	430	+ Staph Spp, E coli
11	28	5	106	4	168	442	+ Stapf Spp, Strep Spp
12	30	7	113	4	176	452	++ Staph Spp, Strep SPP
13	22	6	105	4	168	438	++ E coli, Staph spp
14	21	8	98	3	140	412	+ Staph Spp, Pasurellaspp
15	25	8	102	4	165	435	++ Staph Spp, Strep spp
16	21	9	96	3	132	406	+ Staph Spp, Strep Spp
17	26	7	86	3	128	406	++ Strep Spp, Pasturellaspp
18	21	8	95	3	137	410	++ Staph Spp, Pasturellaspp
19	24	8	108	4	171	441	++ Staph Spp, E coli
20	26	6	98	4	161	433	+ Staph Spp, Ecoli

Appendix II. Different reproductive parameters of Lugol`s iodine infused on 5 days PP in cross-bred dairy cows.

Serial No	UI	First DF	FO	RSC	DO	CI	*Remarks
1	18	7	42	1	42	312	+ + +++ Staph spp, Strepto spp
2	16	8	45	1	45	317	++++ +Strept sp, Pasturella mol., E. coli
3	20	8	46	1	46	321	++++ Strept spp, Paturella mot spp, E.coli
4	17	7	50	1	50	328	++++ Stahp Spp, Strep spp,
5	18	6	48	1	48	318	++++ Strept spp, E. coli
6	17	7	40	1	61	333	+++++ Srept spp, Staph spp
7	15	7	46	1	46	321	++++ Staph spp, Strept spp
8	16	6	55	1	55	326	++++ Strep spp, E. coli
9	17	6	45	1	45	320	++++ +strept spp, E. Coli
10	18	7	48	1	48	318	++++ Staph spp, Strep spp
11	17	8	30	2	72	344	++++ Strept spp, Staph spp, E. Coli
12	15	6	48	1	48	323	++++ Staph spp, E.coli
13	21	8	53	1	53	323	++++ Staph spp, Strept spp
14	16	7	35	1	56	326	++++ Srept spp, E.coli
15	18	6	21	1	42	318	++++ Corny spp E.coli
16	17	8	36	1	57	336	++++ Straph spp, E coli
17	18	7	42	1	42	312	++++ Srepto spp. E. Coli
18	15	7	36	1	57	329	++++ Staph spp, Strept spp
19	18	6	55	1	55	325	++++ +Strept spp, E. coli
20	16	6	32	2	74	347	++++ Staph spp, Strept spp

*** Bacterial count determined before treatment**

Exp. II: Control

Serial No.	UI	First DF	FO	RSC	DO	CI	Remarks
1	**25**	**10**	**102**	**3**	**146**	**421**	+++ Stahp spp, strept spp, E. coli
2	**28**	**8**	**95**	**3**	**137**	**408**	++ Staph spp, Strept spp, Coryn spp
3	**30**	**9**	**120**	**4**	**183**	**453**	+++ Strept spp, Coryn spp, E. coli
4	**28**	**8**	**135**	**3**	**177**	**452**	+++ Coryn spp, E. Coli
5	**28**	**7**	**140**	**6**	**245**	**517**	+++ Strept spp, E. coli
6	**30**	**8**	**118**	**4**	**181**	**451**	+++ Coryn spp, E. Coli
7	**30**	**9**	**125**	**4**	**188**	**460**	++ Staph spp, Strept spp , E .coli
8	**25**	**11**	**98**	**4**	**161**	**436**	+++ Straph spp, E. coli
9	**32**	**7**	**132**	**4**	**195**	**465**	+++ Strep spp, Coryn spp
10	**25**	**8**	**140**	**4**	**203**	**473**	+++ Coryn spp, E. coli
11	**28**	**7**	**155**	**4**	**218**	**490**	+++ Staph spp, Strept spp, E. Coli
12	**26**	**12**	**142**	**4**	**205**	**481**	+++ Coryn spp, E. Coli
13	**35**	**9**	**128**	**3**	**170**	**440**	+++ Staph spp, Strept spp
14	**26**	**8**	**121**	**3**	**163**	**435**	+++Strept spp, Coryn spp, E. coli
15	**28**	**10**	**121**	**3**	**163**	**433**	+++ Staph spp, Coryn spp, E. Coli
16	**28**	**9**	**160**	**4**	**223**	**498**	++ Strep spp, E.coli
17	**25**	**8**	**152**	**4**	**215**	**491**	+++ Staph spp, Strept SPP, E. Coli
18	**30**	**7**	**110**	**5**	**194**	**466**	+++ Strept spp, E. Coli
19	**32**	**9**	**98**	**3**	**140**	**415**	++ Staph spp, E. Coli
20	**21**	**11**	**115**	**4**	**178**	**448**	+++ Corny SPP, E. Coli

Appendix III. Different reproductive parameters of Povidone iodine infused on day 5 PP in cross-bred dairy cows

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Serial No	UI	First DF	FO	RSC	DO	CI	*Remarks
1	12	7	50	1	50	325	+++ Staph spp Strepspp,E coli
2	11	7	45	1	45	317	+++ Stah spp, E. Coli
3	14	6	48	2	69	345	++ Staph spp, strept spp
4	11	6	42	1	42	312	+++ Strep Spp, E coli
5	14	7	45	1	45	317	+++ Strep Spp, Staph spp. E coli
6	13	7	55	1	55	330	+++ Strep Spp, E coli
7	12	6	60	2	81	361	+++ Strep Spp, Staph, E coli
8	13	7	55	1	55	331	++++ Srep Sp, Staph spp , E coli
9	14	7	45	2	66	341	+++ Srep Spp, Stapg, E coli
10	14	6	48	1	48	318	+++ Staph Spp, Strep Spp, E coli
11	15	8	46	1	46	318	+++ + Staph Spp, Strep Spp, E coli
12	14	6	48	2	69	339	++++ Staphspp, StrepsppE coli
13	12	7	45	1	45	321	+++ Srep Spp, Staph Spp
14	12	6	42	2	63	333	++++ Strep Spp Staph Spp, Paturellaspp
15	14	7	46	1	46	321	++++ Srep Spp, Staph Spp
16	15	6	48	1	48	320	++++ Step Spp, Staph Spp, E coli
17	14	7	50	1	50	332	++++ Staph Spp, E coli
18	11	7	63	1	63	337	++++ Staph Spp, E coli
19	14	8	45	1	45	320	++++ Staph Spp, Strep Spp
20	12	6	50	1	50	325	++++ Strep Spp , Pasturella Spp

*** Bacterial count determined before treatment**

Appendix III Control

Serial No	UI	First DF	FO	RSC	DO	CI	Remarks
1	28	7	95	4	158	328	+++ Staph spp Strepspp,E coli
2	28	6	110	4	173	448	+++ Stah spp, Coryn Sp, E. Coli
3	26	10	120	3	162	432	++ Staph spp, strept spp
4	30	7	115	4	178	450	+++ Strep Spp, E coli
5	25	9	106	3	148	422	+++ Strep Spp, Staph spp. E coli
6	28	6	125	4	188	466	++ Strep Spp, E coli
7	21	6	118	3	160	430	+++ Strep Spp, Staph, E coli
8	28	7	122	4	185	438	+++ Srep Sp, Staph spp , E coli
9	25	9	132	3	174	442	+++ Srep Spp, Stapg, E coli
10	21	8	98	3	140	412	+++ Staph Spp, Strep Spp, E coli
11	28	8	122	3	164	436	++ + Staph Spp, Strep Spp, E coli
12	25	8	117	3	169	439	++ Staphspp, Coryn sppE coli
13	25	9	114	3	156	431	+++ Srep Spp, Staph Spp
14	30	11	120	3	162	437	+++ Strep Spp Staph Spp, Paturellaspp
15	21	7	116	5	200	470	+++ Srep Spp, Staph Spp
16	26	6	125	3	167	442	+++ Step Spp, Staph Spp, E coli
17	30	7	95	5	178	450	+++ Staph Spp, E coli
18	28	8	120	4	183	458	++ Staph Spp, E coli
19	28	12	110	4	173	451	++ Staph Spp, Strep Spp
20	30	8	123	3	165	441	++ Strep Spp , Pasturella Spp

Appendix IV. Days open, rate of service per conception and CI of cross-bred dairy cows infused with Lugol`s iodine pre-insemination

Serial No	DO	RSC	CI	*Remarks
1	125	1	401	++++ Staph spp Strepspp,E coli
2	140	1	416	++++ Stah spp, Strept spp,Coryn Sp
3	115	1	387	++++ Staph spp, strept spp
4	160	1	435	++++ Strep Spp, E coli
5	145	1	425	++++ Strep Spp, Staph spp. E coli
6	98	1	371	++++ Strep Spp, E coli
7	110	1	384	++++ Strep Spp, Staph, E coli
8	135	1	411	++++ Srep Sp, Staph spp , E coli
9	120	1	398	+++ Srep Spp, Stapg, E coli
10	118	1	388	+++ Staph Spp, Strep Spp, E coli
11	128	1	398	+++ + Staph Spp, Strep Spp, E coli
12	155	1	427	++++ Staphspp, Strept sppE coli
13	123	1	398	+++ Srep Spp, Staph Spp
14	130	1	406	++++ Strep Spp Staph Spp, Paturellaspp
15	152	1	428	++++ Srep Spp, Staph Spp
16	160	1	435	++++ Step Spp, Staph Spp, E coli
17	155	1	431	++++ Staph Spp, E coli
18	165	1	435	++++ Staph Spp, E coli
19	128	1	400	++++ Staph Spp, Strep Spp E.Col;i
20	148	1	424	++++ Strep Spp , Pasturella Spp, E. Coli

*** Bacterial count determined before treatment**

Appendix IV. Days open, rate of service per conception and CI of cross-bred dairy cows infused with Lugol's iodine post-insemination

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Serial	DO	RSC	CI	* Remarks
1	125	1	392	++++ Staph spp Strepspp,E coli
2	140	2	386	+++ Stah spp, Strep spp, Coryn Sp
3	115	1	387	++++ Staph spp, strept spp
4	160	2	415	++++ Staph spp, Strep Spp, E coli
5	145	1	400	++++ Strep Spp, Staph spp. E coli
6	98	1	390	++++ Strep Spp, E coli
7	110	2	454	++++ Strep Spp, Staph, E coli
8	135	1	419	++++ Srep Sp, Staph spp , E coli
9	120	2	426	+++ Srep Spp, Stapg, E coli
10	118	1	426	+++ Staph Spp, Strep Spp, E coli
11	128	2	462	+++ + Staph Spp, Strep Spp, E coli
12	155	1	407	++++ Staphspp, Coryn sppE coli
13	123	2	415	+++ Srep Spp, Staph Spp
14	130	1	415	++++ Strep Spp Staph Spp, Paturellaspp
15	152	1	434	++++ Srep Spp, Staph Spp
16	160	1	420	++++ Step Spp, Staph Spp, E coli
17	155	2	461	++++ Staph Spp, E coli
18	165	1	445	++++ Staph Spp, E coli
19	128	1	431	++++ Staph Spp, Strep Spp
20	148	2	471	++++ Strep Spp , Pasturella Spp, E. Coli

*** Bacterial count determined before treatment**

Appendix IV. Control

Serial	DO	RCS	CI	Remarks
1	145	5	401	++++ Staph spp Strepspp,E coli
2	150	5	504	+++ Stah spp, Coryn Sp
3	120	4	415	++++ Staph spp, strept spp
4	115	6	490	++++ Strep Spp, E coli
5	180	5	513	++++ Strep Spp, Staph spp. E coli
6	175	5	489	++++ Strep Spp, E coli
7	145	6	512	++++ Strep Spp, Staph, E coli
8	110	4	448	++++ Srep Sp, Staph spp , E coli
9	125	5	479	+++ Srep Spp, Stapg, E coli
10	102	4	440	+++ Staph Spp, Strep Spp, E coli
11	115	5	470	+++ + Staph Spp, Strep Spp, E coli
12	135	6	510	++++ +Staphspp, Coryn sppE coli
13	120	5	476	+++ Srep Spp, Staph Spp
14	112	5	466	++++ Strep Spp Staph Spp, Paturellaspp
15	145	4	484	++++ Srep Spp, Staph Spp
16	165	4	438	++++ +Step Spp, Staph Spp, E coli
17	155	5	510	++++ Staph Spp, Pasurella spp, E coli
18	180	4	513	+++++ Staph Spp, E coli
19	142	4	480	++++ Staph Spp, Strep Spp
20	145	5	499	++++ Strep Spp , Pasturella Spp

Appendix V. Days open, rate of service per conception and CI of cross-bred dairy cows infused with Povidone iodine pre-insemination

Serial No.	DO	RSC	CI	*Remarks
1	95	1	371	++++ Staph spp Strepspp,E coli
2	131	2	407	+++ Stah spp, Strept spp
3	146	2	416	++++ Staph spp, strept spp
4	98	1	370	++++ Strep Spp, E coli
5	130	1	402	++++ Strep Spp, Staph spp. E coli
6	122	1	398	++++ Strep Spp, E coli
7	109	1	386	++++ Strep Spp, Staph, E coli
8	136	2	416	++++ Srep Sp, Staph spp , E coli
9	128	1	404	+++ Srep Spp, Stapg, E coli
10	135	1	409	+++ Staph Spp, Strep Spp, E coli
11	167	2	447	+++ + Staph Spp, Strep Spp, E coli
12	176	2	449	++++ Staphspp, Strept sppE coli
13	136	1	406	+++ Srep Spp, Staph Spp
14	124	1	396	++++ Strep Spp Staph Spp, Paturellaspp
15	106	1	379	++++ Srep Spp, Staph Spp
16	138	1	413	++++ Step Spp, Staph Spp, E coli
17	193	2	455	++++ Staph Spp, E coli
18	163	1	430	++++ Staph Spp, E coli
19	142	1	414	++++ Staph Spp, Strep Spp
20	121	1	391	++++ Strep Spp , Pasturella Spp

*** Bacterial count determined before treatment**

Appendix V. Days open, rate of service per conception and CI of cross-bred dairy cows infused with Povidone iodine post-insemination

Serial No.	DO	RSC	CI	*Remarks
1	141	2	419	++++ Staph spp Strepspp,E coli
2	119	2	391	+++ Stah spp, Coryn Sp
3	133	2	403	++++ Staph spp, strept spp
4	157	2	428	++++ Strep Spp, E coli
5	149	2	424	++++ Strep Spp, Staph spp. E coli
6	183	2	462	++++ Strep Spp, E coli
6	163	2	439	++++ Strep Spp, Staph, E coli
7	190	3	465	++++ Srep Sp, Staph spp , E coli
8	155	1	427	+++ Srep Spp, Stapg, E coli
9	198	2	470	+++ Staph Spp, Strep Spp, E coli
10	132	1	406	+++ + Staph Spp, Strep Spp, E coli
11	157	2	420	++++ Staphspp, Coryn sppE coli
12	140	2	410	+++ Srep Spp, Staph Spp
13	104	1	374	++++ Strep Spp Staph Spp, Paturellaspp
14	194	3	474	++++ Srep Spp, Staph Spp
15	191	2	468	++++ Step Spp, Staph Spp, E coli
16	187	2	457	++++ Staph Spp, E coli
17	98	1	370	++++ Staph Spp, E coli
18	103	1	373	++++ Staph Spp, Strep Spp
19	143	2	414	++++ Strep Spp , Pasturella Spp
20	156	2	432	++++ Staph spp, Strept spp, E. Coli

*** Bacterial count determined before treatment**

Appendix V Control

Serial No.	DO	RSC	CI	Remarks
1	264	5	534	++++ Staph spp Strepspp,E coli
2	212	5	487	+++ Stah spp, Coryn Sp
3	216	4	488	++++ Staph spp, strept spp
4	279	5	536	++++ Strep Spp, E coli
5	204	5	479	++++ Strep Spp, Staph spp. E coli
6	250	6	525	++++ Strep Spp, E coli
7	265	6	537	++++ Strep Spp, Staph, E coli
8	218	4	490	++++ Srep Sp, Staph spp , E coli
9	222	5	496	+++ Srep Spp, Stapg, E coli
10	251	6	524	+++ Staph Spp, Strep Spp, E coli
11	226	6	496	+++ + Staph Spp, Strep Spp, E coli
12	200	5	476	++++ Staphspp, Coryn sppE coli
13	269	5	541	+++ Srep Spp, Staph Spp
14	224	5	494	++++ Strep Spp Staph Spp, Paturellaspp
15	237	6	509	++++ Srep Spp, Staph Spp
16	161	4	438	++++ Step Spp, Staph Spp, E coli
17	196	5	469	++++ Staph Spp, E coli
18	228	6	503	++++ Staph Spp, E coli
19	211	4	485	++++ Staph Spp, Strep Spp
20	221	4	492	++++ Strep Spp , Pasturella Spp