

## The Role of Improper Use of Artificial Insemination on Infertility and Abortion in Cows in Basrah Province, Iraq: Microbiological Study

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### دور التلقيح الاصطناعي غير المناسب على العقم والإسقاط في البقر في منطقة البصرة – العراق: دراسة ميكروبيولوجية

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دراسة استشرافية أجريت لإظهار المسببات المعدية وطرق انتقالها في حالات العقم والإسقاط في الأبقار. لقد اجري التلقيح الاصطناعي إلى 434 بقرة، وقد فشل الحمل في 155 (35.7%) حالة منها بعدة عدة محاولات من التلقيح الاصطناعي. وكذلك فان 70 بقرة تم تلقيحها بالطريقة الطبيعية، وقد فشل الحمل في 30 (42.8%) حالة منها. وكذلك وجد تأخر الدورة التروية بعد الولادة الطبيعية في 398 (99%) من مجموع 402 بقرة. لقد تم عزل عدة أحياء مجهريه معروفة بتسببها للعقم والإسقاط في الأبقار من عينات الرحم والسائل المنوي المجمد ومن أجهزة التلقيح الاصطناعي. تم عزل جرثومة باستوريليا ايروجير لمفردها من عينة رحم لبقرة واحدة بعد الإجهاض مباشرة. وكذلك فان هذه الجرثومة تم عزلها من ثلاث عينات من السوائل المنوية المجمدة ومن إحدى أجهزة التلقيح الاصطناعي. يستنتج من هذه إن هذه الجرثومة غير مسجلة سابقاً بكونها تسبب الإسقاط أو العقم. الدراسة بأن الجراثيم المرضية ممكن أن تنتقل إلى الأبقار السليمة بواسطة أجهزة التلقيح الاصطناعي الملوثة أو بواسطة السائل المنوي الملوثة. لذلك فإنه يوصي بإجراء زرع جرثومي روتيني وفحص مجهري مباشر للسائل المنوي الذي يستخدم للتلقيح الاصطناعي مع استثناء أو علاج الثيران التي تظهر فيها الإصابة بالأمراض. وكذلك تعقيم أجهزة التلقيح الاصطناعي بصورة روتينية قبل كل استعمال.

Key words: Abortion in cows, Artificial insemination, Cows, Infertility, Microbiological study

#### ABSTRACT

A prospective study was performed to demonstrate the infectious causes and their mode of transmission in cases of infertility and abortion in cows. There were 434 artificially inseminated cows, of which 155 (35.7%) did not conceive after several trials. Seventy cows were naturally inseminated, of which 30 (42.8%) did not conceive. Delayed oestrus after normal delivery was found in 398 (99%) of 402 cows. Many microorganisms, known to be causative agents of infertility and abortion in cows,

were recovered from uterine specimens, frozen semen and instruments of artificial insemination (AI). *Pasteurella aerogenes* alone was isolated from a uterine culture of one aborted cow. This organism was also isolated from three samples of frozen semen and from an instrument of AI. This microorganism has not been reported previously as a causative agent of abortion or infertility.

It is concluded that the recovered pathogens could be transmitted from contaminated AI instruments or from frozen semen to healthy cows. Therefore, it is recommended to make routine culture and direct microscopical examination of semen that are used for AI and excluding or treating the infected bulls. Sterilization of AI instruments should also be performed routinely before each use.

## Introduction

Artificial insemination, which has been available in developed and many developing countries for several decades, provided a major advance in animal breeding [1].

Economically, abortion is of great concern to the farmer, because the fetus is lost; a prolonged period of uterine disease and sterility may follow; the unproductive female must be maintained for a long period or sold, and if the cause of the abortion is infection, it threatens the rest of the herd.

If artificial insemination is practiced without regard for diseases transmission, however, it can result in transmission of infectious organisms more rapidly than any other way [2].

The aim of this study was to demonstrate the infectious causes of infertility and abortion with special emphasis on their methods of transmission by AI instruments and nitrogen-frozen semen.

## Materials And Methods

A prospective study extended from January 1992 to May 1997 including 776 cows which were collected from a private clinic, and from January 1996 to May 1997 including 434 cows which were collected from a government center.

During the period of October 1996 to May 1997, uterine specimens were cultured from 11 cows with abortion or infertility. Direct wet preparation for *Trichomonas fetus* was made for 21 cows with abortion or infertility. Cultures of 19 samples of nitrogen-frozen semen were performed. In addition, cultures from all parts of AI instruments (catheter, body of gun, blade, piston and gun) were done for 10 government centers and four private clinics, i.e. all AI centers in Basrah.

The specimens for culture were inoculated on 5% sheep blood agar, MacConkey agar and Chocolate agar (under 5-10% carbon dioxide incubation). Bacterial isolates were identified using conventional methods [3].

## Results

There were 434 cows (of which 32 were heifer) inseminated artificially, of which 155 (35.7%) did not conceive after several AI (infertility). While delayed oestrus (subfertility) after normal delivery was found in 398 (99%) of 402 cows (32 heifer were excluded) (Table 1). Failure rate of AI of 776 cows in a private clinic is shown in (Table 2).

In the region of Hartha, there was a gradual decreasing in the number of artificially inseminated cows during the period of 1990-1996. This might reflect a decrease in the number of cows in that area (Figure 1).

*Pseudomonas aeruginosa* was the most common organism (5 isolates) recovered from uterine cultures of 11 cows (45.4%) with infertility or frequent abortion (Table 3). *Trichomonas fetus* was also isolated from 8 (38%) of 21 cows with infertility or frequent abortion.

There were 19 cultures of nitrogen-frozen semen, of which 9 (47.4%) revealed positive bacterial cultures. The most frequent organism isolated was *Pasteurella aerogenes* (Table 3).

Surface swab cultures from all parts of AI instruments were made. *Corynebacterium pyogenes* was the most frequent organism isolated from these instruments (Table 3).

*Pasteurella aerogenes* alone (monomicrobial culture) was isolated from uterine specimen culture of aborted cow after six months pregnancy. This microorganism was also isolated from three samples of frozen semen, and from one instrument of AI (Table 3).

### Discussion

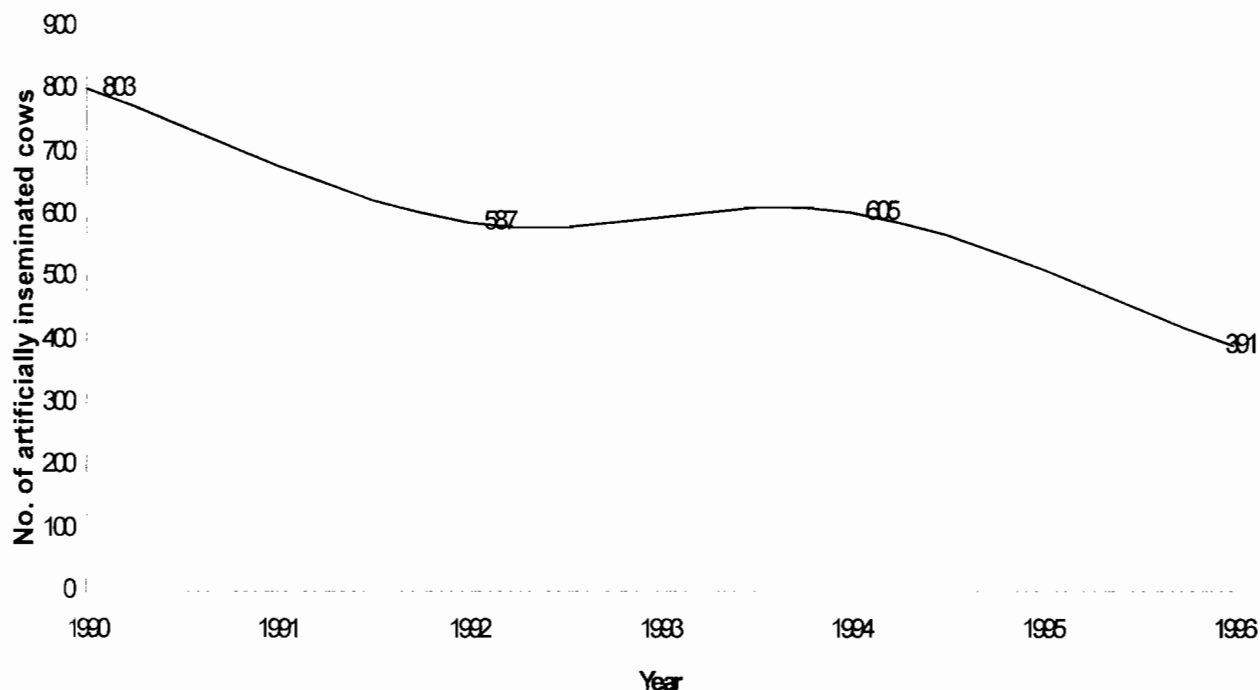
In the present study, the infertility was recognized in 35.7% of cows after several AI and in 42.8% of cows after natural insemination, while delayed oestrus (subfertility) was found in 99% of cows. This rate of infertility is higher than those reported by Dessouky and Juma [4] in Iraq that the infertility was 10.1%, Vale et al. [5] in Brazil (30%), Bloomfield et al. [6] in U.K. (6%), and Cavestany and Galina [7] in Uruguay (12.5%). However, the high rate of infertility in the present study might be the cause of decline in the number of cows in Hartha area as shown in Figure 1. Postpartum anoestrus has been divided into 'true anoestrus' when there is no ovarian activity and 'false anoestrus' when there is follicular activity ovulation and corpus luteum formation but no oestrus [8]. Although true anoestrus is related to energy deficiencies [9,10] false anoestrus is generally occasioned by problems in oestrus detection [11]. Energy

**Table 1. Time of oestrus after normal delivery of 402 cows during the year 1996 in the government center of Hartha.**

First oestrus after normal delivery Month	No. of cows (%)
1.3 (normal)	4 (1)
2	29 (7.2)
3	37 (9.2)
4	70 (17.4)
5	64 (16)
6	83 (20.6)
7	38 (9.4)
8	30 (7.4)
9	6 (1.5)
10	7 (1.7)
11	0 (0)
12	34 (8.4)
<b>Total</b>	<b>402 (100)</b>

**Table 2. The failure rate of artificial insemination of 776 cows during the period of 1992-1996 (from private clinic).**

Year	No. of artificially inseminated cows	No. (%) of conception failure
1992	185	23 (12.4)
1993	90	3 (3.3)
1994	90	1(1.1)
1995	157	12 (7.6)
1996	254	81 (31.9)
<b>Total</b>	<b>776</b>	<b>120 (15.5)</b>



**Figure 1. Decline of artificially inseminated cows during the period of 1990-1996 in the government center of hartha**

**Table 3. Organisms isolated from cultures of uterine specimens, nitrogen-frozen semen and instruments of artificial insemination.**

Isolates	Number of Isolates		
	Uterine specimen	Nitrogen-frozen semen	Instruments of AI
*Pseudomonas aeruginosa	5	0	5
*Staphylococcus aureus	2	0	11
*Staphylococcus albus	2	0	4
*Haemophilus sp.	2	2	0
Pasteurella aerogenes	1	3	1
*Pasteurella multocida	0	1	1
*Nocardia asteroides	1	0	0
*Proteus sp.	1	0	5
Klebsiella sp.	1	0	12
Lactobacillus sp.	1	0	0
*Listeria monocytogenes	0	2	0
Enterobacteriaceae	0	2	0
Enterobacter sp.	0	1	0
*Streptococcus sp.	0	1	2
*Corynebacterium pyogenes	0	0	15
Bacillus sp.	0	0	5
*Escherichia coli	0	0	1
*Yeast	0	0	6
<b>Total</b>	<b>16</b>	<b>12</b>	<b>68</b>

\*Previously reported to be a causative agent of abortion and/or infertility.

deficiencies might play a role as a cause of infertility in some cases in the present study.

Many organisms which were recovered in our study from uterine specimens of cows with infertility or abortion and/or from frozen semen and/or instruments of AI that were also reported to be causative agents of abortion and infertility in cows in previous studies [12,13,14,15,16,17]. These organisms included: *Trichomonas fetus*, *Corynebacterium pyogenes*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus albus*, *Nocardia asteroides*, *Escherichia coli*, *Listeria monocytogenes*, *Pasteurella multocida*, *Proteus* sp., *Haemophilus* sp., *Streptococcus* sp. and Yeast. Nevertheless, these organisms could be transmitted to healthy cows either by contaminated instruments of AI or by frozen semen. Therefore, meticulous sterilization of instruments of AI, and routine direct microscopical examination and bacteriological culture of bull's semen, which are used for AI process, is mandatory. The infected bulls should be treated or excluded from the procedure of semen collection. In human, viruses such as HIV-1 infection was reported to be transmitted by AI [18], and hepatitis C virus was detected from donor semen [19], and the bacteria *Chlamydia trachomatis*, *Mycoplasma hominis*, *Ureaplasma urealyticum*, *S. aureus* and group B Streptococci were also detected in the seminal fluids of the donors [20].

*Pasteurella aerogenes* alone was recovered as monomicrobial culture from a uterus of aborted cow after six months conception. This microorganism was also isolated from frozen semen culture of three samples and from many parts of one AI instrument. This might indicate a significant role of this organism in causing abortion or infertility. This organism, however, was not reported as a causative agent of abortion or infertility in previous studies.

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