



Using Sexed Semen to Produce Homo-Sexual Twins from Dairy Friesian Cows Under Egyptian Condition

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Abstract

THE presented study was conducted to investigate the impact of vaginal and uterine pH on the efficiency of fertilization with sexed semen of dairy Friesian cows. Thirty Friesian anestrus cows were used and divided into three groups (10 each). The uteri of cows in the two groups was washed with pH buffer solution, being 6 for 1st group, and 8 for 2nd group, while the 3rd group not washing and kept as a control group. All groups were subjected to a Cosynch+progesterone protocol to induce estrus, and ultrasound scanning, and blood samples were taken at various intervals. This study results found that adjusting the pH level had no significant effect on ovarian follicle development, but it had a significant effect on the rate and growth of dominant follicles in the acidic groups. Finally, adjusting the pH of the vagina and uterus during insemination to 6 for X- sexed semen and 8 for Y- sexed semen increases semen efficiency, and resulting in identical twins of 60% for AC and 40% for AK compared to 10% for conventional semen.

Keywords: Friesian dairy cows; Double ovulation; Sexed semen; Vaginal and uterus pH.

Introduction

Livestock resources are the cornerstone of global food security and the agricultural economy. Dairy cows, particularly Friesians, are pivotal in meeting the demand for dairy products. In the ongoing quest to improve production and reproductive efficiency, artificial insemination techniques have emerged as essential tools for developing the dairy industry. One of the most prominent is artificial insemination using sexed semen, which allows for very high control over the offspring sex. This rapid economic development is beneficial to breeders, particularly for replacing female or male animals (1). Despite significant advancements in semen sorting technologies, the efficiency of fertilization and pregnancy rates using sex-sorted semen can be slightly lower compared to conventional semen (2). In this context, the physiological factors of the female reproductive environment, most notably the pH of the uterus and vagina, become crucial. The pH plays a vital role in sperm survival and motility. It is believed that adjusting the pH level to suit the desired chromosome type (acidic for X, alkaline for Y) can

enhance the fertilization efficiency of sex-sorted semen (3). Furthermore, inducing double ovulation represents a promising strategy to increase the chances of fertilization and pregnancy, thereby boosting herd productivity. By stimulating the ovary to produce two oocytes instead of one in a single cycle, the opportunities for sperm to fertilize ova are doubled. This could potentially compensate for any marginal decrease in the efficiency of sex-sorted semen or increase twinning rates, which can maximize economic returns (4). Based on the foregoing, this research aims to explore the feasibility of enhancing the efficiency of sex-sorted semen through an integrated approach that combines optimizing the uterine and vaginal pH to match the chromosomal type in the sorted semen and implementing a hormonal program to induce double ovulation. The impact of these interventions will be evaluated on pregnancy success rates, offspring sex, twinning rates, and calving ease, with the ultimate goal of developing more effective and efficient artificial insemination protocols that contribute to promoting sustainable animal production.

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Material and Methods

This study was carried out at El-Karada Experimental Station, Kafr El-Sheikh province belonging to the Animal Production Research Institute (APRI), Agriculture Research Center, Egypt during the period from December to July (2022).

This study was performed on 30 multiparous medium lactating Friesian cows with different ages (4 ± 1.5 years) and weights (500 ± 25 kg). The experimental animals were divided after waiting voluntary period into 3 groups (10 animals in each group) as follows: First group: Animals were washing vaginally and uterine with slightly acidic (pH 6) buffer solution (Bedaya Center of Fertility and IVF, Giza, Egypt). Second group: Animals were washing vaginally and uterine with a slightly alkaline (pH 8) buffer solution (Bedaya Center of Fertility and IVF, Giza, Egypt). The third group: the uterus and vagina of the animal were not washed and kept as a control. All groups were given a Cosynch + progesterone synchronization protocol: at Day 0: cows were received an intramuscularly injection of 100 µg GnRH (Receptal; Intervet MSD Animal Health Ltd, Egypt SAE) and intra-vaginal Controlled Intravaginal Drug Release (CIDR) device inserted from the beginning day till day 6 of treatment (1.38 g progesterone, CIDR Cattle Insert; Zoetis New Zealand Limited, Auckland, New Zealand) then at Day 3 cows received an injection of 800 IU eCG intramuscularly (Folligon; MSD Animal Health Ltd), at Day 6 cows were received an intramuscularly injection of 500 µg cloprostenol (PG, Estrumate; MSD Animal Health Ltd., Wellington, New Zealand), in conjunction with CIDR removal. At Day 8 cows were received a second 100 µg GnRH intramuscularly injection in conjunction with insemination (Fig. 1).

Ovarian monitoring: Ovarian structures of cows were monitored at 9:00 a.m. on the sixth and eighth days of the program by ultrasonography (Advanced Digital Ultrasonic Diagnostic Imaging System Model: Dus-6000. Anti-electro-shock Type: Class I 100V-240V ~50HZ/60HZ 150VA; Advanced Inc. Miami, U.S.A) once daily at d 6 and d 8 (ovulation and insemination day). The development in the dominant follicle at injected PG at day 6 in the protocol: time ovulation from injected PG (h), count mature follicles were recorded.

The pH measurements of the uterus and vagina

During oestrus, the external genitalia were cleaned with a mild antiseptic solution and dried with cotton, mucus samples were collected by aspiration using a sterilized glass pipette (10 ml), whose pointed end was connected to a syringe with a rubber junction. The glass pipette was followed per rectally to pass into the cervix or near the vaginal fold. The pH was recorded at the previous calving, before the

oestrus synchronization program, and at insemination.

Serum blood biochemical levels

Blood samples were collected (10 ml) from the jugular vein once daily of all experimental cows at 12:00 a.m. on Day 45 before current calving, serum was separated by centrifugation and stored at -20 °C until analysis to measuring the level of calcium (Mg/dl), phosphorus (Mg/dl), magnesium (Mg/dl), and zinc (µg/dl) using Atomic Absorption spectroscopy assay (AAS).

Reproductive and productive standards

The following criteria were evaluated: Number of services per conception, conception rate, total birth (n and %), type of birth, sex ratio (male n and %: female n and %), total twin birth (n and %), total like-sexed (n and %), birth weight of calves at 1st month, 2nd month and 3rd month, uterine involution time (d), placental drop duration (h) and pregnancy period (d).

Manual examination of the genitalia via rectum was examined in day 10, 15, 22, 25 and 32 postpartum by symmetry of both gravid and non-gravid horns.

Statistical analysis

Data were analyzed by a Completely Randomized Design (CRD) in the case of replicates are equal General linear models, univariate. Data were represented as mean \pm standard deviation values. Least Significant Difference, LSD) test was performed for comparing values among the groups General linear model, univariate $P < 0.05$ was considered to be significant. The obtained data were statistically analyzed [5] using a computer program of IBM Corp [6].

$$\text{Statistical Model} \quad Y_{ij} = \mu + T_i + e_{ij}$$

Y_{ij} : the value of j observation pertaining to the transaction i

μ : The general average of the studied trait.

T_i : The effect of the treatment. I

e_{ij} : The random error is normally distributed with a mean of zero and a variance of σ^2_e

Results

Adjusting the pH of the uterus and vagina before artificial insemination with sexed semen

Average vagina and uterus pH values at previous parturition, during apply synch-protocol, and at insemination. As shown in Table (1), no significant differences were found in the pH of the uterus and vagina between the experimental groups for the previous birth before applying the estrus synchronization program. This is due to the fact that the feeding programs provided to the animals during this period are constant and the pH of the animals'

rumens is constant. The average pH for the previous birth was 7.2 for the AC and Control groups and 7.1 for the AK group, while during the application of the program it was 7.1 for the AC and AK group and 7.2 for the Control group. However, there are significant differences in the pH during the insemination of the animals. This is due to direction of the environment of the uterus and vagina to be AC to suit the sexed semen X (AC group) and alkaline to suit the semen Y (AK group), while the Control group was kept as it was and the average pH was 5.9 for the AC group, 8.4 for the AK group and 7.2 for the Control group.

The impact of the double ovulation synchronization program on the treatments

As shown in Table (2), there were significant differences in the period (d) from the beginning of the program until ovulation between the AC group and the other two groups, where the shortest period was recorded with an average of 7.5 d for the AC group, while average of 9.5 d recorded for the alkaline group, and 9.6 d for the control group. This is probably due to the pH of the uterus and vagina. There were also significant differences in the diameter (mm) of the mature egg between the AC group and the other two groups, where the largest diameter was recorded with an average of 18.93 mm for the AC group, 14.3 mm for the alkaline group, and 13.09 mm for the control group. While the number (n) of mature eggs during ovulation did not differ significantly between the experimental groups, and the largest number of ovulations was recorded with an average of 2.6 oocytes for the AC group, followed by the controls with an average of 2 oocytes. The alkaline group had the lowest average (1.8 oocytes).

Reproductive and productive parameters of offspring

As shown in Table (3), there were significant differences between the three groups in the sex ratio (%) and the percentage of twin births, while there were no significant differences in the percentage of single births and the percentage of matching between the sex of the newborn and the semen. The AC had the highest sex ratio for females and twins and the lowest for singletons out of the total number of births, as twins and singletons were 100% identical to the sex of the sperm they inseminated with, and recorded 37.2%, 2.33% and 9.30%, respectively, out of the total number of births. While the AK had the highest sex ratio for males, as the sex ratio of twin and single births was recorded at 27.9% male - 7.1% female, 18.6% male, 11.6% - male, 2.3% female, respectively, of the total births number, as the twin births were sexually identical with the semen with which they were inseminated at a rate of 100%, while the single births were at a rate of 90%. While the control group had the highest rate of non-identical twins and singleton births and the lowest rate of identical twins: 9.30% male-female, 11.6%

male -4.75% female, 4.65% male, respectively, of the total births number. The high percentage of females in the first group and no males is due to the fact that they were fertilized with sexed semen containing the X chromosome. There is also a relationship between the pH of the uterus and vagina, as the AC pH enhances the vitality and fertilization of the X chromosome and prevents fertilization of the Y chromosome. This is what the results explain. As for the high percentage of fertilization, it is due to the use of a highly efficient multiple ovulation program.

Effect of the pH of the uterus and vagina on some reproductive parameters

The data presented in Table (4) showed the effect of three treatments (AC, AK, and Control) on some variables related to pregnancy and calving in cows. The variables studied included, number of services per pregnancy (n): There appears a slight difference between the three groups, with values of 1.1, 1.3, and 1.1, respectively. Gestation period (d): There were a significant difference between the AC group and the AK and Control groups, being a shorter gestation period in AC group (275.8 days) as compared to the other two groups (285.3 and 282.9 days). Placental abruption time (h): There were no significant differences found between the experimental groups, being 9.2, 10.3, and 8.95 hours for AC, AK and Control groups, respectively. Uterine return time (d): There was a significant difference between the AC group and both AK and Control groups, being longer in the AC group (50 days) compared to the others (38.3 and 34.9 days). Results indicate that the AC treatment has an effect on gestation period and uterine return time, while there is no significant effect on placental abruption time. These results could be important in understanding the effects of different treatments on pregnancy and parturition performance in cows. *Effect of uterine and vagina pH on neonatal weight and growth monitoring*

The data presented in Table (5) indicate the birth weight and growth monitoring of neonates in three different groups: AC, AK, and Control. There was no significant difference between the groups in the weight of singletons, birth weight after the first and second month, daily growth rate, and weaning weight. Birth weight of twins: There was a significant difference between the groups, as the AK group had significantly higher birth weights compared to the AC and Control groups, with averages of 28.26, 26.33, and 26.03, respectively. The AK group had significantly higher twin birth weights compared to the AC and Control groups. The difference in twin birth weights between the groups could indicate a different effect of the factor being studied (AC and AK) on the growth of twins.

Levels of some minerals in prenatal blood serum

The data presented in Table (6) indicate blood levels of calcium, phosphorus, magnesium, and zinc in three different groups: AC, AK, and Control. Calcium: There is a significant difference between the groups, the AK group having significantly higher calcium levels than the AC group. The Control group did not differ significantly from the other two groups. Phosphorus, magnesium, and zinc: There are no significant differences between the groups, with phosphorus levels being similar in all three groups.

Discussion

Based on research on sperm motility within the reproductive tract, it has been observed that varying pH levels may affect the activity of sperm carrying both X and Y chromosomes. For example, some studies have shown that alkaline buffers (with a higher pH) increase the proportion of Y chromosome sperm in the upper layer of semen samples, while AC buffers (with a lower pH) have been associated with a higher proportion of X chromosome sperm [7]. In agreement with research regarding sperm survival in the reproductive tract, it has been shown that extreme pH values (AC and alkaline) can negatively affect sperm motility and viability, which may hinder their ability to reach and fertilize the mature ovum. Regarding sperm migration, studies have shown that changing the pH of cervical mucus can affect the upward migration rate of sperm carrying the Y chromosome [8]. Agreeing with some studies regarding the effect of sex ratio and specific pH ranges, it has been shown that an alkaline vaginal pH (high pH) is associated with a higher incidence of male fetuses. Conversely, an AC vaginal pH (low pH) is associated with a higher incidence of female foetuses [9]. In some studies, specific pH ranges have been associated with different sex ratios. For example, a pH range of 7.5 to 8.3 was associated with a higher incidence of males, while a pH range of 6.5 to 7.3 was associated with a predominance of females [3].

Dual ovulation synchronization program, also known as a double stimulation protocol, aims to increase the number of oocytes retrieved during ovulation. While it doesn't directly alter the diameter of a mature egg, it can lead to more mature eggs being available for use, potentially increasing the number of fertilized oocytes [10]. Dual stimulation: This technique involves stimulating the ovaries with gonadotropins (like FSH or human menopausal gonadotropin; hMG) for an extended period, leading to the development of multiple follicles and oocytes. Increased oocyte yield: Studies have shown that dual stimulation can increase the number of oocytes retrieved in a single ovarian cycle, compared to a single stimulation protocol [11]. Not directly affecting oocyte size: While dual stimulation focuses on increasing the number of oocytes, it does not

directly impact the size or quality of the individual oocyte [12]. Potential for increased fertilization: The increased number of oocytes available for fertilization can potentially lead to higher fertilization rates and a greater number of embryos [12]. Improved fertilization and pregnancy outcomes: By increasing the number of oocytes and embryos, dual stimulation protocols can potentially improve pregnancy rates in certain cases. In summary, a dual ovulation synchronization program can increase the number of eggs retrieved during ovulation, which may ultimately lead to more fertilized Oocytes and improved pregnancy outcomes [4].

The results indicate a relationship between the type of chromosome in the semen and the sex of the offspring. The first group (X-chromosome) produced only females, while the second group (Y-chromosome) produced mostly males. These results are consistent with previous studies that showed which the sex of the offspring depends on the type of chromosome in the semen [13]. Results also indicate that the pH of the uterus may influence the sex of the offspring. The first group (X-chromosome) and a pH of 5.9 produced only females, while the second group (Y-chromosome) and a pH of 8.4 produced mostly males. These results are consistent with previous studies that showed that the pH of the uterus can affect the motility and activity of sperm, which may affect the sex of the offspring [3]. A study on dairy Holstein cows found that double ovulation can increase the chances of pregnancy and improve the fertilization efficiency of sexed semen in cows. These results are consistent with previous studies and underscore the importance of understanding the factors that influence pregnancy and fertility in cows [14]. The relationship between double ovulation and sex ratio in cattle inseminated with sex-sorted semen has been studied to understand how these factors affect pregnancy rates and sex ratios of offspring. Research suggests that using sex-sorted semen in double ovulation protocols can lead to more successful pregnancies and potentially influence the sex ratio of calves born. [15].

Vaginal pH was found to affect the number of inseminations in cows, in cows with more alkaline vaginas needing fewer inseminations to achieve pregnancy [17]. Some studies have found that uterine pH affects the number of times that cows can be inseminated, in cows with more acidic uteri requiring more inseminations to achieve pregnancy [16]. Study of [18] suggested that cervical mucus pH is a key factor in determining conception success in cattle, as cervical mucus is the transport medium for sperm in the female reproductive tract. The [19] study reported that a pH between 7.0 and 8.5 is optimal, supporting sperm viability and motility, while a pH below 6 results in poor motility. Study of [20] found that the mean pH of cervical and vaginal mucus in un-breeding cows was 7.40 ± 0.09 and 7.95 ± 0.09 .

In a recent report, [21] suggested that the mean pH values of cervical mucus in pregnant and non-pregnant cows were 8.30 ± 0.11 and 7.76 ± 0.12 , respectively. The percentage of conceived cows that had thin vaginal mucus during estrus was significantly ($P < 0.05$) higher 87.5% compared to those with thick vaginal mucus [22]. Most studies indicate that uterine and vaginal pH play an important role in determining the time to placenta expulsion and the return of the uterus to its normal position after calving in cows. It is important to note that uterine and vaginal pH can be influenced by many factors, including the mother's overall health and nutrition. A study on Friesian cows found that uterine pH affected the time to placenta expulsion after calving, in cows with a more acidic uterus having a longer time to placenta expulsion [23]. Another study found that vaginal pH affected the return of the uterus to its normal position after calving in cows, with a more alkaline vagina having a faster return of the uterus to its normal position [24]. Meanwhile, a study on dairy Holstein cows concluded that uterine and vaginal pH affected uterine health and the return of the uterus to its normal position after calving [25]. Most studies indicate that uterine pH plays an important role in determining calving weight in cows and can impact fetal growth and health. However, the precise mechanisms of this effect are still not fully understood and require further research. In agree with a study by [26] which found that uterine pH affects calf weight in cows, as cows with more acidic uterine gave birth to lighter calves. Also in agree with a study by [27] who found that the pH of the uterus affects fetal growth in cows with a more alkaline uterus gave birth to heavier offspring. [28] found that the pH of the uterus affected the health of the newborn cows, with more acidic uteruses gave birth to offspring more susceptible to disease. The normal weight of twin calves in cows varies depending on breed of cow. This finding is supported by a study conducted on Holstein cows, which found that the normal weight of twin calves is approximately 25-35 kg per calf [29]. Consistent with another study conducted on Friesian cows, it was found that the normal weight of twins is approximately 20-30 kg per newborn [30]. A study conducted on Holstein cows found that the normal weight of twin calves is approximately 15-30 kg per calf, and this can be affected by several factors, including the breed of cow and the breeding of the cows [31]. These studies indicate that the normal birth weight of twin calves in cattle ranges from 15-35 kg per calf and can be influenced by many factors. It's important to note that low birth weight for twin calves can increase the risk of death or disease, while excess weight can increase the risk of birth complications. It's worth noting that twin calves in cattle are often lighter than single calves and can be more susceptible to health problems. Therefore, it's

important to carefully assess the birth weight of twin calves to ensure they receive appropriate care. Contrary to a study conducted on Holstein cows, the normal weight of a singleton calf is found to be approximately 40-45 kg [32]. Based on another study conducted on Friesian cows, the normal weight of a singleton calf in beef cattle is found to be approximately 35-40 kg [33]. Consistent with a study conducted on Holstein cows, the normal weight of a singleton calf in beef cattle is found to be approximately 30-50 kg [34] and can be influenced by many factors, including the breed and breeding of the cow. These studies indicate that the normal weight of a singleton calf in beef cattle ranges from 30-50 kg and can be influenced by many factors.

The normal range of calcium cations in the blood before calving in cows is approximately 8-10 mg/dL [35]. Another study found that the normal range of phosphorus cations in the blood before calving in cows is approximately 4-7 mg/dL [36]. On the other hand, a study on Friesian cows found that the normal range of magnesium cations in the blood before calving in cows is approximately 2-3 mg/dL [37]. A study conducted on Holstein cows in the journal found that the normal range for zinc cations in the blood before calving in cows is approximately 0.8-1.2 mg/L [38]. It is important to note that these ranges can vary slightly depending on the study, breed, and other factors. Changes in the levels of these cations can also be an indicator of health problems in cows.

Conclusion

Adjusting the pH of the vagina and uterus during insemination to 6 for X- sexed semen and 8 for Y- sexed semen increases the efficiency of the semen, to produce Homo-Sexual Twins of 60% for AC and 40% for AK compared to 10% for conventional semen.

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

This experiment was conducted according to the guide-lines of Kafrelsheikh University and approved by the local experimental animal care committee, Faculty of Agriculture, Kafrelsheikh University, Egypt (id 4/2016 Ec). All precautions were taken to decrease the risk of injury and disease throughout the trial period.

TABLE 1. Average vagina and uterus pH values at previous parturition, during apply synch-protocol, and at insemination in the experimental groups.

Item	AC	AK	Control
pH for previous parturition	7.2 ± 0.32	7.1 ± 0.6	7.2 ± 0.4
pH during apply synch-protocol	7.1 ± 0.31	7.1 ± 0.2	7.2 ± 0.3
pH during insemination	5.9 ± 0.41 ^a	8.4 ± 0.2 ^b	7.2 ± 0.3 ^c

a, b, c: Different letters on the same row indicate significance ($P < 0.05$), analyzed by Completely Randomized Design (CRD) in the case of replicates are equal – General linear models, univariate.

TABLE 2. Average time (day) to ovulate for tow ovaries since the synchronization protocol start, mature oocytes diameter (mm) and mature oocytes number (n).

Item	AC	AK	Control
Period (d) from GnRH injection and ovulation	7.5 ± 0.4 ^a	9.5 ± 0.6 ^b	9.6 ± 0.5 ^b
Mature oocytes diameter (mm)	18.93 ± 1.3 ^a	14.30 ± 2.5 ^b	13.09 ± 1.1 ^b
Mature oocytes number (n)	2.6 ± 0.54	1.8 ± 0.83	2.0 ± 0.70

a, b: Different letters on the same row indicate significance ($P < 0.05$), analyzed by Completely Randomized Design (CRD) in the case of replicates are equal – General linear models, univariate.

TABLE 3. Percentage (%) of reproductive and productive characteristics of offspring.

Item	AC	AK	Control	Total births number
Cows number	10	10	10	30
Total birth (n)	16	13	14	43
Sex ratio (%)				
Male	0 ^c (0)	27.9% ^b (12)	23.25% ^a (10)	22
Female	37.2% ^a (16)	2.33% ^c (1)	9.3% ^b (4)	21
Twins birth %				
Male	0 ^c (0)	18.6% ^a (8)	4.65% ^b (2)	10
Female	27.9% ^a (12)	0 ^b (0)	0 ^b (0)	12
Male+ Female	0 ^b (0)	0 ^b (0)	9.3% ^a (4)	4
Single birth %				
Male	0 ^b (0)	11.6% ^a (4)	11.6% ^a (6)	10
Female	9.3% ^a (4)	2.3% ^c (1)	4.75% ^a (2)	7
Total Like-sexed (%)				
Like	100%	90%	100%	
Unlike	0 ^b	10% ^a	0 ^b	

a, b, c: Different letters on the same row indicate significance ($P < 0.05$), analyzed by crosstabs-Chi-Square Test of Independence in the case of replicates are equal – descriptive statistic, multivariate.

TABLE 4. Effect of the pH of the uterus and vagina on some reproductive parameters.

Item	AC	AK	Control
Number of services per conception (n)	1.1	1.3	1.1
Gestation period (d)	275.8 ± 23.4 ^b	285.3 ± 37.1 ^a	282.9 ± 59.5 ^a
Placental abruption time (h)	9.2 ± 1.74	10.3 ± 1.96	8.95 ± 1.75
Uterine involution time (d)	50.0 ± 4.9 ^a	38.3 ± 1.5 ^b	34.9 ± 5.7 ^b

a, b: Different letters on the same row indicate significance ($P < 0.05$), analyzed by Completely Randomized Design (CRD) in the case of replicates are equal – General linear models, univariate.

TABLE 5. Effect of the pH of the uterus and vagina on the weight (kg) of the newborn for single and twin birth.

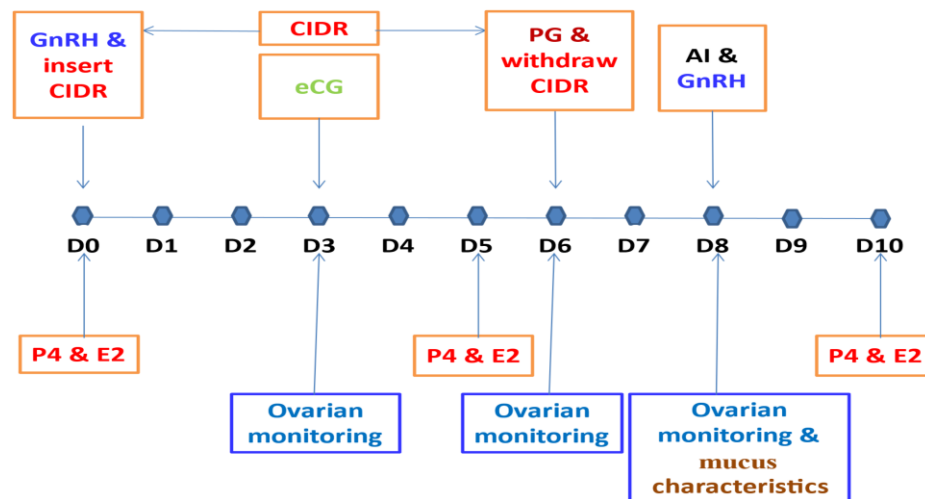
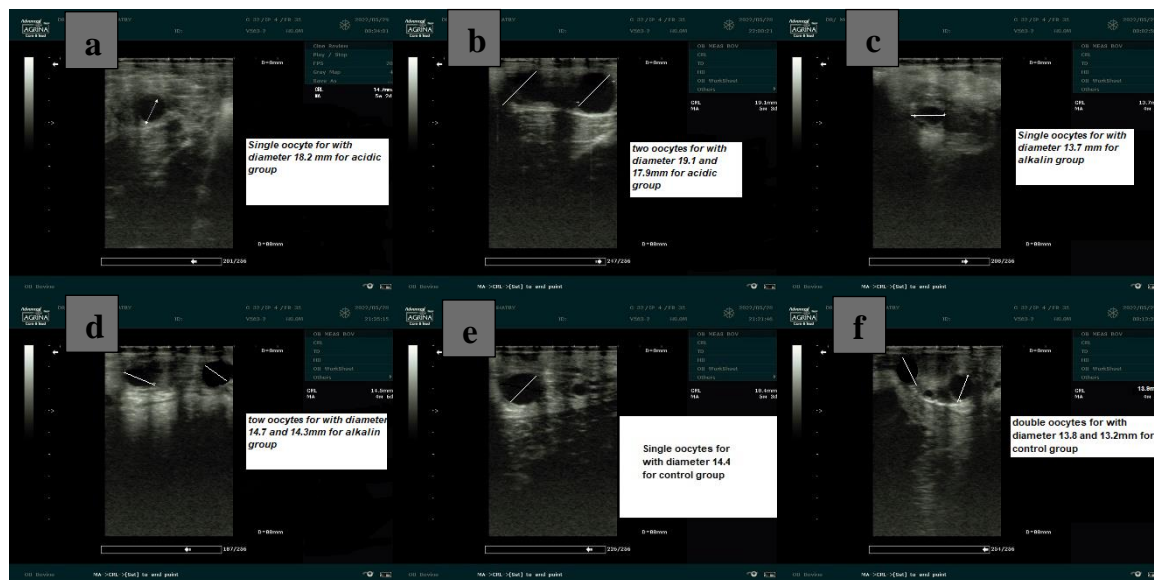
Item	AC	AK	Control
Single birth			
Number of births (n)	4	5	7
Birth weight (kg)	30.10 ± 3.78	30.40 ± 3.94	31.40 ± 3.71
Twin birth			
Number of births (n)	6	5	3
Birth weight (kg)	26.33 ± 3.23 ^c	28.26 ± 3.54 ^b	26.03 ± 3.16 ^c
Single and Twin			
Number of calves (n)	10	10	10
Weight after the first month (kg)	51.30 ± 6.75	53.40 ± 7.10	51.70 ± 6.61
Weight after the second month (kg)	65.50 ± 8.88	68.60 ± 9.10	66.20 ± 8.49
Average daily growth rate (g/d)	650	672	669
Weaning weight (kg)	106.4 ± 15.13	107.6 ± 15.79	111.6 ± 14.87

a, b: Different letters on the same row indicate significance ($P < 0.05$), analyzed by Completely Randomized Design (CRD) in the case of replicates are equal – General linear models, univariate.

TABLE 6. Average of some mineral elements in blood serum two months before birth the in experimental groups.

Item	AC	AK	Control
Calcium (Mg/dl)	8.02 ± 0.44 ^b	8.57 ± 0.21 ^a	8.26 ± 0.41 ^{ab}
Phosphorus (Mg/dl)	3.98 ± 0.28	4.01 ± 0.36	3.92 ± 0.39
Magnesium (Mg/dl)	2.63 ± 0.33	2.70 ± 0.26	2.80 ± 0.29
Zinc (µg/dl)	104.3 ± 10.49	106.0 ± 11.80	109.0 ± 10.06

a, b, c, d: Different letters within the same hormone indicate significance ($P < 0.05$)

**Fig. 1.** Hormonal treatment protocol and timing of samples collection and ovarian monitoring**Fig. 2.** Average diameter of mature oocyte (mm) before insemination. a, ultrasound snapshot showing the single mature oocyte (mm) for acidic group ; b, ultrasound snapshot showing the double mature oocyte (mm) for acidic group ; c, ultrasound snapshot showing the single mature oocyte diameter (mm) for alkaline; d, ultrasound snapshot showing the double mature oocyte diameter (mm) for left alkaline group; e, ultrasound snapshot showing the single mature oocyte diameter (mm) for control group ; f, ultrasound snapshot showing the double mature oocyte diameter (mm) for control group.

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استخدام السائل المنوي المجنس لإنتاج توائم متماثلة الجنس من أبقار الفريزيان الحلوب تحت الظروف المصرية

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الملخص

تناولت هذه الدراسة تأثير درجة الأس الهيدروجيني للمهبل والرحم على تراكيب المبيض لدى أبقار الفريزيان الحلابة. وقد تم استخدام ثلاثين بقرة فريزيان عديمة الشبق وتم تقسيمها إلى ثلاث مجموعات (10 لكل مجموعة). تم غسل أرحام الأبقار في المجموعتين بمحلول منظم لدرجة الأس الهيدروجيني، 6 درجة في المجموعة الأولى، و8 درجة في المجموعة الثانية، والمجموعة الثالثة لم يتم غسلها وتم الاحتفاظ بها كمجموعة تحكم. خضعت جميع المجموعات لبروتوكول Cosynch + progesterone لتحفيز الشبق، والمسح بالموجات فوق الصوتية، وتم أخذ عينات الدم على فترات مختلفة. وجدت الدراسة أن إن تعديل درجة حموضة المهبل والرحم أثناء التلقيح إلى 6 للسائل المنوي ذي الجنس X و8 للسائل المنوي ذي الجنس Y يزيد من كفاءة السائل المنوي، لإنتاج توائم متماثلة الجنس بنسبة 60% للسائل المنوي ذي الجنس AC و40% للسائل المنوي AK مقارنة بـ 10% للسائل المنوي التقليدي.

الكلمات الدالة: درجة الأس الهيدروجيني للمهبل والرحم ، الحويصلات المبيضية ، تنظيم الشياخ ، أبقار الفريزيان الحلابة.