

Pregnancy Success in Bitches - Evaluation of Interactions between Artificial Insemination Method, Serum Progesterone Concentration and Vaginal Cytology Parameters

Margarita Martínez-Mojica^{1,3}, Adrián Guzmán², Ana María Rosales-Torres²,
José Antonio Herrera-Barragán², Héctor Castillo-Juárez^{2*} & Cyndi Gabriela Hernández-Coronado²

ABSTRACT

Background: The artificial insemination has become a well-established method in the breeding of bitches, and evaluation of the factors that may potentially affect pregnancy success is essential. For this reason, it is essential to evaluate the factors that may affect fertility of the bitch when artificial insemination is performed. Serum progesterone concentrations and vaginal cytology have been used to determine the time of ovulation and stage of the estrus cycle. This study aimed to evaluate the artificial insemination method, the serum progesterone concentration, the breed size, age, the whelping number, vaginal cytology parameters, and their interactions on pregnancy success in bitches.

Materials, Methods & Results: A total of 607 bitches that had undergone reproductive consultation with the Mexican Canine Federation from January to December 2016 were enrolled in the present study and assigned to one of 2 artificial insemination methods (intravaginal and transcervical) using fresh semen. Determination of the estrus cycle phase and the time of Artificial insemination was based on vaginal cytology and serum progesterone concentrations. Bitches inseminated by the transcervical technique had a higher pregnancy rate with respect to females inseminated by the intravaginal technique ($P < 0.05$). Moreover, females with a serum progesterone concentration of 5-10 ng/mL had a greater probability (> 4 times) of getting pregnant than animals with lower or higher progesterone concentrations ($P < 0.05$). Bitches inseminated by the intravaginal technique and with serum progesterone concentrations > 10 ng/mL had a considerable reduction in pregnancy ($P < 0.05$) compared with females with < 10 ng/mL serum progesterone or with bitches inseminated by the transcervical technique.

Discussion: Serum progesterone concentration, the artificial insemination method, and superficial cells without a nucleus modified the pregnancy rate in bitches. Females inseminated by transcervical semen deposition had a higher pregnancy rate than females inseminated by the intravaginal technique. Using fresh or frozen-thawed semen produced a higher pregnancy rate in bitches inseminated by transcervical semen deposition than females inseminated by the intravaginal technique. Differences in the pregnancy rate between transcervical and intravaginal insemination could be associated with the correct semen disposition, the distance that the sperm must travel to reach the oocyte, as well as the number of sperm that reach the oviduct ampulla. Exist evidences that after ovulation, as progesterone rises, the cervix is closed, which may compromise the passage of the sperm deposited into the vagina. Therefore, it is likely that in females with a serum progesterone concentration > 10 ng/mL, the cervix was closed, compromising the ability of the sperm to access the oviduct. Thus, the use of intravaginal insemination should be done in bitches with a serum progesterone concentrations < 11 ng/mL to reduce the possibility of cervical closure and to increase the odds of pregnancy. It is well documented that the serum progesterone concentration and vaginal cytology parameters have a great influence on pregnancy success, and the results confirm these findings. In the present study, 96% of the bitches inseminated with a serum progesterone concentration of 5-10 ng/mL got pregnant and had higher odds of pregnancy than bitches with lower or higher serum progesterone concentrations.

Keywords: female dogs, reproductive performance, pregnancy rate, fertility, intravaginal, transcervical, fresh, semen.

DOI: 10.22456/1679-9216.127693

Received: 6 October 2022

Accepted: 21 December 2022

Published: 17 January 2023

¹División de Ciencias Biológicas de la Salud & ²Departamento de Producción Agrícola y Animal, Universidad Autónoma Metropolitana-Xochimilco (UAM-Xochimilco), Ciudad de México, Mexico. ³Federación Canófila Mexicana, Ciudad de México. ⁴In memoriam. CORRESPONDENCE: C.G. Hernández-Coronado [chernandezc@correo.xoc.uam.mx]. Departamento de Producción Agrícola y Animal, UAM-Xochimilco. Calzada del Hueso n. 1100. CP 04960 Ciudad de México, Mexico.

INTRODUCTION

Artificial insemination (AI) with fresh or frozen semen has been widely used in dogs [12,14] and has become a powerful reproductive tool for bitch breeding [8,12]. For this reason, it is essential to evaluate the factors that may affect fertility of the bitch when AI is performed.

Serum progesterone concentrations and vaginal cytology have been used to determine the time of ovulation and stage of the estrus cycle, and their association with pregnancy success is well established [1,5,7,11]. However, other factors such as the insemination method have produced contradictory results. In a study where using frozen-thawed semen, observed that the pregnancy rate was reduced in bitches inseminated by laparotomy (45%) compared with bitches inseminated by transcervical semen deposition (65%) [9]. On the other hand, the pregnancy rate was higher in bitches inseminated by transcervical semen deposition compared with bitches inseminated by the intravaginal technique [3]. In contrast, in a study, authors did not find a difference in the pregnancy rate between bitches inseminated by intravaginal or laparotomy semen deposition [13]. Thus, the present study aimed to evaluate the effect of the AI method, the serum progesterone concentration, the breed size, age, the whelping number, vaginal cytology parameters, and their interactions on pregnancy success in bitches.

MATERIALS AND METHODS

Animals

All animal management was performed according to the protocols for the care and welfare of animals, stipulated in the Official Mexican Regulation 062-ZOO-1999. A total of 607 bitches that had undergone reproductive consultation with the Mexican Canine Federation from January to December 2016 were enrolled in the present study and assigned to 1 of 2 AI methods using fresh semen. The mean age of the bitches was 34 ± 17 months, and the population evaluated consisted of animals from small (French Bulldog, Norwich Terrier, Pug and Miniature Schnauzer; 38%), medium (English Bulldog; 82%), and large (Dogue de Bordeaux and Golden Retriever; 45%) breeds. The whelping number varied from 1 to 5.

Progesterone concentrations

Determination of the estrus cycle phase and the time of AI was based on vaginal cytology and serum progesterone concentrations. All the bitches were

brought to the clinic for examination around 5 days after the onset of serosanguinous vulvar discharge noted by the owner; cytology was performed¹. The cell types for each animal were identified using an optical microscope with the 10× objective lens. The cell types recorded were parabasal, intermediate, superficial with a nucleus, superficial without a nucleus, and erythrocytes [15]. The quantity of each cell type was classified as abundant when it was > 70%, regular when it was between 30% and 70%, and scarce when it was < 30%. Cytology was performed every 3rd day until superficial cells without a nucleus were > 70%. Then, daily blood samples were collected via jugular or cephalic venipuncture until the serum progesterone concentration was ≥ 4 ng/mL. Blood was collected into vacutainer tubes without additives and centrifuged at 1000 g for 10 min to obtain serum. Progesterone was determined immediately after serum collection by chemiluminescence². AI was designed to be performed only 1 time when the serum progesterone concentration was 4-10 ng/mL for intravaginal insemination and 10-20 ng/mL for transcervical insemination. This range of progesterone represents the entire period of fertility in a bitch [9]. However, it is important to clarify that even when this criterion was supposed to be used for all the bitches that arrived for a reproductive consultation, some females were inseminated with lower or higher progesterone concentrations, due to the owners' convenience.

Semen collection

For fresh semen collection, males were held gently by the neck, while performing physical contact on the back and the abdomen, to desensitize them. The prepuce was retracted using a latex glove to expose the penis up to the posterior side of the bulb glands. Constant pressure was applied to the caudal part of the penis, posterior to the bulb glands, to induce an erection and to allow the penis to rotate in the caudal direction. The pressure was continued to maintain the erection and to induce ejaculation [15]. The ejaculate was collected into 15 mL tubes, avoiding urine contamination.

Insemination

Was performed using a concentration of live morphologically normal sperm $> 90 \times 10^6$ and progressive motility of $\geq 80\%$. These parameters were verified via the computer-assisted semen analysis system³.

Intravaginal AI (n = 188) was performed using a 20 mL plastic pipette, which was inserted approximately

3-10 cm into the vagina depending on the animal's size. The semen was deposited into the vagina using a 20 mL syringe. The bitches were placed with their hind limbs raised for 10 min, and it was recommended to the owner to walk the bitch for 10 min after the insemination without letting her sit and urinate [15]. Transcervical AI (n = 419) was performed according to method previously described [9], using a rigid ureterorenoscope⁴ equipped with a xenon cold light source; it was introduced through the vagina to visualize the cervix. A CH-5 transcervical catheter³ was passed through the ureterorenoscope and the cervix. When the tip of the catheter was in the uterus body, the semen was deposited slowly over a period of 10 min. Pregnancy diagnosis was made by ultrasonography at day 30 after AI⁵ with a sectorial probe of 3.5 MHz.

Statistical analysis

Were carried out using JMP-13 statistical software⁶. The effects of the AI method, the progesterone concentration, and their interaction were determined by logistic regression. For this, the bitches were divided into 3 groups based on serum progesterone: 5-10 ng/mL, which represents the period from the onset to the end of ovulation, < 5 ng/mL, and > 10 ng/mL. Later, were analyzed the main effects, the AI method, the progesterone concentration, the breed size, the whelping number, age, and superficial cells without a nucleus on the pregnancy success by using logistic regression, and was calculated the odds of pregnancy success. The progesterone concentrations and vaginal smear used for analysis were the last values collected prior to AI. The progesterone concentration (ng/mL) was used as a covariate to determine the effect of the insemination method on the pregnancy rate. Additionally, was evaluated the interactions between the insemination method, the breed size, superficial cells without a nucleus, and the whelping number on the pregnancy rate.

RESULTS

There was no interaction between the AI method and the serum progesterone concentration ($P > 0.05$). However, both the AI method and the serum progesterone concentration modified the pregnancy rate (Table 1; $P < 0.05$). Bitches inseminated by transcervical semen deposition had a higher pregnancy rate and were 4.8 times more likely to become pregnant than bitches inseminated by the intravaginal technique. Bitches with a serum progesterone concentration between 5 and 10 ng/mL had a higher pregnancy rate ($P < 0.05$) than bitches inseminated with a serum progesterone concentration of < 5 or > 10 ng/mL.

Moreover, bitches inseminated with a serum progesterone concentration between 5 and 10 ng/mL had a 7.5- and 5-times greater probability of getting pregnant compared with bitches with a serum progesterone concentration of > 10 or < 5 ng/mL, respectively. Finally, the pregnancy rate was higher ($P < 0.05$) in bitches with an abundant number of superficial cells without a nucleus compared with bitches with a scarce number but not compared with bitches with a regular number of these cells (Table 1). The odds of pregnancy were 3.7 time higher in bitches with an abundant number of superficial cells without a nucleus compared with bitches with a scarce number. The pregnancy rate was not affected by age, the breed size, or the whelping number, or their interaction with the AI method ($P > 0.05$).

Finally, was determined the effect of the AI method within each progesterone range as well as the effect of progesterone for each AI method (Figure 1). Bitches inseminated by the transcervical or intravaginal technique had a higher pregnancy rate when their serum progesterone concentration was 5-10 ng/mL compared with bitches with serum progesterone of < 5 or > 10 ng/mL. However, for the transcervical technique there was not a difference ($P < 0.05$) in the pregnancy rate between bitches with a serum progesterone concentration of < 5 or > 10 ng/mL. In contrast, bitches inseminated by the intravaginal technique with a serum progesterone concentration < 5 ng/mL had a higher pregnancy rate ($P < 0.05$) than bitches with a serum progesterone concentration > 10 ng/mL.

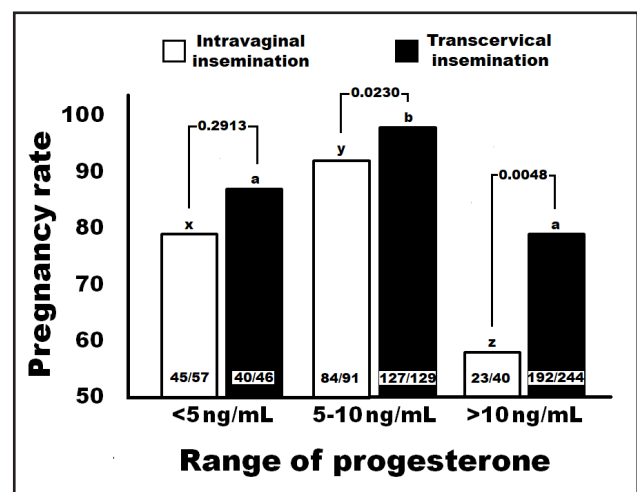


Figure 1. Effect of the serum progesterone concentration and the artificial insemination technique on the pregnancy rate in bitches. a & b - Indicates statistical differences by progesterone range in bitches inseminated by transcervical technique. x, y, & z - Indicate statistical differences by progesterone range in bitches inseminated by the intravaginal technique. [Bars with a common superscript are not different at $P < 0.05$].

Table 1. Effect of the artificial insemination (AI) technique, the serum progesterone concentration, the breed size, the whelping number, and superficial cells without a nucleus on the pregnancy rate of bitches.

	n	Pregnancy rate (%)	95% CI	Odds	95% CI	P
<i>AI technique</i>						
Intravaginal	188	152 (81) ^b	75 - 87	1		
Transcervical	419	359 (86) ^a	82 - 89	4.8	2.7 - 8.8	< 0.0001
<i>Range of progesterone concentration</i>						
> 10 ng/mL	284	215 (76) ^a	71 - 81	1		
5 - 10 ng/mL	220	211 (96) ^b	96 - 99	7.5	3.7 - 15.5	< 0.0001
< 5 ng/mL	103	85 (83) ^a	75 - 90	1	0.9 - 2.7	0.1574
5 - 10 ng/mL	220	211 (96) ^b	96 - 99	5.0	2.111.5	0.0002
<i>Breed size</i>						
Small	118	98 (83)	76 - 90	1		
Medium	205	178 (87)	82 - 91	1.4	0.7 - 2.5	0.3387
Large	284	236 (83)	79 - 87	1	0.6 - 1.8	0.9627
<i>Whelping number</i>						
Nulliparous	183	153 (84)	78 - 89	1		
Primiparous	66	59 (85)	76 - 94	1.1	0.5 - 2.4	0.8138
Multiparous	31	27 (87)	75 - 100	1.3	0.4 - 4.1	0.624
<i>Superficial cells without a nucleus</i>						
Scarce	69	27 (69) ^a	54 - 84	1		
Regular	55	49 (84) ^{ab}	74 - 94	2.3	0.8 - 6.1	0.103
Abundant	291	260 (89) ^b	86 - 93	3.7	1.7 - 8.1	0.0009

CI: confidence interval. ^{a,b}superscript letters indicate significant differences in the pregnancy rate for each factor ($P < 0.05$).

DISCUSSION

In the present study, the results shown that the serum progesterone concentration, the AI method, and superficial cells without a nucleus modified the pregnancy rate in bitches. Bitches inseminated by transcervical semen deposition had a higher pregnancy rate than bitches inseminated by the intravaginal technique. Previous studies have reported similar results: Using fresh or frozen-thawed semen produced a higher pregnancy rate in bitches inseminated by transcervical semen deposition than bitches inseminated by the intravaginal technique [2,3,10]. Consistently with [10] differences in the pregnancy rate between transcervical and intravaginal insemination could be associated with the correct semen disposition, the distance that the sperm must travel to reach the oocyte, as well as the number of sperm that reach the oviduct ampulla.

Bitches with a serum progesterone concentration > 10 ng/mL had a lower pregnancy rate than bitches with a serum progesterone concentration

of 5-10 ng/mL no matter the insemination method. However, it is important to note that this reduction in the pregnancy rate was more pronounced in bitches inseminated by intravaginal semen deposition. Whereas the reduction in the progesterone range was only 20% in bitches inseminated by the transcervical technique, the reduction was 37% in bitches inseminated by the vaginal method. This result is explained by evidence that after ovulation, as progesterone rises, the cervix is closed, which may compromise the passage of the sperm deposited into the vagina [6,16]. Therefore, it is likely that in bitches with a serum progesterone concentration > 10 ng/mL, the cervix was closed, compromising the ability of the sperm to access the oviduct. Thus, the use of intravaginal insemination should be done in bitches with a serum progesterone concentrations < 11 ng/mL to reduce the possibility of cervical closure and to increase the odds of pregnancy.

It is well documented that the serum progesterone concentration and vaginal cytology parameters have a great influence on pregnancy success, and

these results confirm these findings [5,12,14]. In the present study, 96% of the bitches inseminated with a serum progesterone concentration of 5-10 ng/mL got pregnant and had higher odds of pregnancy than bitches with lower or higher serum progesterone concentrations. These results are explained because over 95% of the bitches have ovulated when their serum progesterone concentration reaches 5-8 ng/mL [1,6,10]. Regarding cytology, $\geq 70\%$ of vaginal epithelial cells without a nucleus could be associated with ovulation [2,4,5]. Thus, in the present study bitches with abundant superficial cells without a nucleus ($> 70\%$) could be ovulating, increasing the probabilities of pregnancy.

CONCLUSION

In conclusion, these results confirm that transcervical semen deposition is better than intravaginal semen deposition to get bitches pregnant and that the

serum progesterone concentration and vaginal cytology are the main factors that determine the pregnancy rate in bitches subjected to AI.

MANUFACTURERS

¹Hycel - Reactivos Químicos. Zapopan, Jalisco, Mexico.

²Siemens Health Care. Erlangen, Germany.

³Minitube USA Inc. Verona, WI, USA.

⁴KARL STORZ Endoscopia México S.A de C.V. Ciudad de México, Mexico.

⁵Fujifilm SonoSite Inc. Bothell, WA, USA.

⁶SAS Institute Inc. Cary, NC, USA.

Acknowledgments. The authors are thankful to Marcia Castillo for proofreading the manuscript.

Ethical approval. The study was conducted in accordance with the protocols for the care and welfare of animals, stipulated in the Official Mexican Regulation 062-ZOO-1999.

Declaration of interest. The authors report no conflicts of interest. The authors alone are responsible for the contents and writing of the paper.

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