

## FACTORS AFFECTING HASTEN OF REPRODUCTIVE AGE IN TAURINE AND ZEBUINE HEIFERS

## Factores que afectan la aceleración de la edad reproductiva en vaquillas taurinas y cebuinas

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### ABSTRACT

It is well established that the timing of the first pregnancy in heifers has a major influence on their lifetime reproductive performance. However, zebuine heifers typically show delayed puberty with the first calving often occurring at around 36–48 months of age. Several factors affect the reproductive efficiency in heifers, such as weight, age, body condition, uterine development and genetics. The thickness of subcutaneous fat efficiently predicts the risk for the establishment of puberty and pregnancy in 14-month-old Nelore heifers submitted to FTAI. It was found that heifers with greater subcutaneous fat thickness were more likely to achieve puberty and to become pregnant at FTAI. Through adequate nutritional management combined with genetic selection for sexual precocity, it is possible to obtain also satisfactory reproductive efficiency in precocious primiparous. When the basic requirements (mainly nutrition and genetics) are met, the anticipation of the conception of heifers can be successfully established, improving the productivity and profitability of breeding livestock.

Keywords: heifers, FTAI, reproductive performance, zebuine, taurine

### RESUMEN

Está bien establecido la edad a la primera preñez en las vaquillas tiene una gran influencia en su desempeño reproductivo de por vida. Sin embargo, las novillas cebuinas suelen mostrar una pubertad tardía y el primer parto suele ocurrir alrededor de los 36 a 48 meses de edad. Varios factores afectan la eficiencia reproductiva en las vaquillas, como el peso, la edad, la condición corporal, el desarrollo uterino y la genética. El espesor de la grasa subcutánea predice eficientemente el riesgo de establecimiento de pubertad y preñez en vaquillas Nelore de 14 meses de edad sometidas a IATF. Se encontró que las vaquillas con mayor espesor de grasa subcutánea tenían más probabilidades de alcanzar la pubertad y quedar preñadas en IATF. Mediante un manejo nutricional adecuado combinado con la selección genética para la precocidad sexual, es posible obtener también una eficiencia reproductiva satisfactoria en primíparas precoces. Cuando se cumplen los requisitos básicos (principalmente nutrición y genética), se puede establecer con éxito la anticipación de la concepción de vaquillas, mejorando la productividad y rentabilidad de la ganadería de cría.

Palabras clave: vaquillas, IATF, eficiencia reproductiva, zebuinas, taurinas

#### INTRODUCTION

It is known that the largest percentage of the herds maintained in the tropics is composed by zebuine (Bos indicus) or crossbred (Bos taurus x Bos indicus), which have greater tolerance to climatic, nutritional and ectoparasite challenges, especially when exposed to tropical management conditions (Nogueira, 2004; Bó et al., 2003; Sartori and Barros, 2011; Baruselli et al., 2017a). However, zebuine animals reach puberty later when compared to taurine (Abeygunawardena and Dematawewa, 2004; Nogueira, 2004). This fact reflects high age at puberty (approximately three years old) and high age at first calving (approximately 4 years old; CEPEA/USP, 2016).

In this context, reducing the interval between birth and the first calving is one of the great challenges of tropical livestock. It is important to point out that reducing the age at first calving reduces the number of heifers on the property, which generates an increase in productivity per pasture area, and a greater economic return from the activity (Baruselli et al., 2017b). Also, it can be noted that the decrease in age at first calving is also directly related to the decrease in the interval between generations, culminating in the intensification of selection and genetic gain of the herds (Baruselli et al., 2018).

To ensure high reproductive efficiency, it is worth mentioning the use of biotechnologies of reproduction such as fixed-time artificial insemination (FTAI) with semen from bulls of high genetic value, aiming at greater weight at weaning of calves and economic return of the activity (Rhodes et al., 2003; Baruselli et al., 2004; Bó et al., 2007).

It is known that there are several factors that affect the efficiency of FTAI in heifers, such as weight, age, body condition, uterine development, and paternity (genetic). Another relevant factor is the re-conception of these heifers when primiparous. These factors will be discussed in this review. In addition, the impact on farm productivity with the reduction of the interval between birth and first calving in beef heifers will be addressed.

## FACTORS THAT INTERFERE IN THE PREGNANCY RATE TO THE FTAI OF YOUNG HEIFERS

#### Zootechnical parameters and sexual precocity

The selection of replacement heifers to start their reproductive life is essential for productivity of the cow-calf operation system (Patterson et al., 2000; Lamb, 2013). For a heifer to be able to reproduce, adequate growth is necessary, combined with the accumulation of body energy reserves (Docchio et al., 2019). Some zootechnical parameters such as age, weight, body condition score (BCS), subcutaneous fat thickness (SCFT) and cDEPTH (height from floor to sternum area immediately caudal to the forelimbs) / hWITHERS = metric ruler installed on theside of the restraining trunk that measured the distance from the ground beneath the animal to the top of the withers directly above the centre of shoulder) x 100] were studied by Freitas (2015) in an attempt to analyze the influence of heifer development on fertility at FTAI. It was observed that heifers with higher age, weight, BCS and SCFT had a higher pregnancy rate at FTAI (Table 1).

The increase in pregnancy rate in older heifers is probably due to a decline in estrogen's negative feedback on the hypothalamus and an increase in GnRH/LH secretion (Anderson et al., 1996). Data from scientific studies showed that body weight when evaluated in isolation has low accuracy to predict fertility, as it does not present a high correlation with the accumulation of subcutaneous fat (Ayres et al., 2009). The authors argue that body weight may be more associated with the size of the animal (Rocha et al., 2003). However, unlike weight, BCS is a parameter that has a high correlation with the animal's body reserves (Ayres et al., 2014), and may be positively related to conception rates at FTAI.

 Table 1. Conception rate at FTAI of Nelore heifers with a mean age of 13.8 months, according to the variables studied

Variable	n	Conception rate	P Value	
Age				
> 13 months	391	40.9%	0.01	
$\leq$ 13 months	259	27.8%	0.01	
Body weight				
> 248 kg	394	39.8%	0.001	
≤ 248 kg	255	29.4%	0.001	
BCS (Dia -10)				
> 3.00	249	45.4%	< 0.001	
≤ 3.00	400	29.7%	< 0.001	
SCFT				
> 2.47 mm	378	44.4%	< 0.001	
≤ 2.47 mm	265	23.4%	< 0.001	
cDEPTH				
> 44.0%	358	41.9%	< 0.001	

In the same study, it was also verified that heifers that had 1 to 3 developmental characteristics above the cut-off point had a higher pregnancy rate at FTAI compared to those that had characteristics below the cut-off point (28.3 vs. 39.7%). Furthermore, when all parameters that were above the cut-off point were added together (age, weight, BCS, height at the withers, subcutaneous fat, cDEPTH), there was an increase in the pregnancy rate of precocious heifers of 28.3% for 47.0%. These data demonstrate the importance of controlling these parameters for the success of a reproductive program in 14month-old Nelore heifers.

#### SUBCUTANEOUS FAT THICKNESS AND SEXUAL PRECOCITY

Nutrition is one of the main factors that interfere in the formation of adipose and muscle tissue. Leptin, a protein hormone originating from adipose tissue, acts in reproductive endocrine control, acting as a peripheral signal for the hypothalamus (Docchio et al., 2019). According to Ebling and Cronin (2000), there is a gradual increase in the hypothalamic expression of GnRH from birth to puberty, an essential condition for the transition from immaturity to sexual maturity. Currently, it is also known that the kisspeptin peptide acts as the main regulator of the GnRH-secreting neuron. The activation of the hypothalamic-pituitary-gonadal axis during the period of sexual maturation promotes the tonic increase of GnRH and LH (Macedo, 2011; Colli, 2018). Leptin is believed to regulate

reproductive function through adipokinins that act on kisspeptinsecreting neurons (Hausman et al., 2012; Docchio et al., 2019).

Fat deposition in the carcass is an important variable for animal growth, since it is determined by the individual's precocity (Suguisawa, 2002). According to Williams et al. (1995), body fat deposition is different between genetic groups. Small animals are usually precocious because they start the process of fat deposition earlier. Furthermore, younger animals have less subcutaneous fat deposition and greater intramuscular fat deposition (Alberta Agricultural Food and Rural Development, 2001). Considering these studies, carcass ultrasound to evaluate body fat deposition can help in the selection of animal precocity.

The assessment of the thickness of subcutaneous fat in the croup or rib presents high accuracy to estimate body energy reserves in cattle (Schroder and Staufenbiel, 2006; Ayres, 2008; Ayres et al., 2009). Freitas et al., (2021) demonstrated that the thickness of subcutaneous fat efficiently predicts the risk for the establishment of puberty and pregnancy in 14-month-old Nelore heifers submitted to FTAI. It was found that heifers with greater subcutaneous fat thickness were more likely to become pregnant at FTAI (Figure 1).

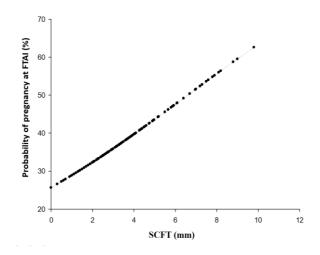


Figure 1. FTAI pregnancy probability as a function of subcutaneous fat thickness in the croup (EGPU) of 643 Nellore heifers with 13.9 months old [Logit(EGPU) = -1.0662 + 0.1612\* PGE; P = 0.0003] (Freitas, 2021).

 Table 4. Effects of improved vs. moderate nutrition on IVEP responses in Nelore heifers with high vs. low genetic selection for sexual precocity.

	Improved	Improved nutrition		moderate nutrition		P value		
Variable °	High genetic	Low genetic	High genetic	Low genetic	Nut	Gen	Nut*Gen	
Number of animals	9	8	11	11				
Viable oocytes, n	23.9	19.9	19.5	20.8	0.69	0.76	0.39	
Viable blastocyst per OPU, n	7.7	3.3	4.0	2.4	0.05	0.009	0.35	
Viable blastocyst rate, %	23.9%	14.7%	23.5%	9.2%	0.11	0.01	0.95	

**Table 5.** Effects of improved vs. moderate nutrition on reproductive performance in Nelore heifers with high vs. low genetic selection for sexual precocity.

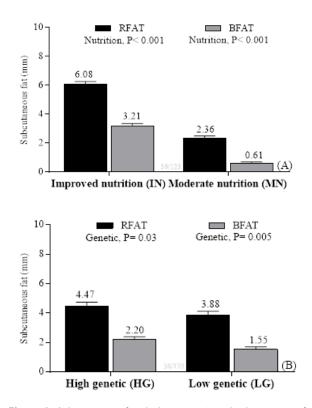
	Improved nutrition		Moderate nutrition		P value		
Variable ª	High genetic	Low genetic	High genetic	Low genetic	Nut	Gen	Nut*Ger
Number of animals	50	47	50	51			
Pregnancy rate at 1 <sup>st</sup> FTAI by doppler, %	66.0% (33/50)	68.1% (32/47)	56.0% (28/50)	39.2% (20/51)	0.007	0.32	0.19
Potential pregnancy lost at 1 <sup>st</sup> FTAI, %	21.2% (7/33)	15.6% (5/32)	14.3% (4/28)	25.0% (5/20)	0.91	0.75	0.28
Effective pregnancy rate at 1 $^{\rm st}$ FTAI, $\%$	52.0% (26/50)	57.5% (27/47)	48.0% (24/50)	29.4% (15/51)	0.02	0.33	0.11
Pregnancy rate at $2^{nd}$ FTAI, %	41.2% (7/17)	26.7% (4/15)	27.3% (6/22)	29.0% (9/31)	0.61	0.57	0.45
Cumulative pregnancy rate, %	66.0% (33/50)	66.0% (31/47)	60.0% (30/50)	47.1% (24/51)	0.07	0.37	0.38

Catussi et al., (2022) evaluated the effects of nutrition and genetic selection for sexual precocity on reproductive performance in young Nelore heifers (14 months) submitted to in vitro embryo production (IVEP) and fixed time artificial insemination (FTAI). A total of 198 heifers were randomly assigned to a  $2 \times 2$  factorial design composed of two nutritional

treatments [Improved nutrition (IN; n=97) and Moderate nutrition (MN; n=101)] and two genetic groups [High genetic (HG; n=100) and Low genetic (LG; n=98)]. After 60 days of the beginning of the experiment, the measurement of subcutaneous fat in both the rib and the croup was performed.

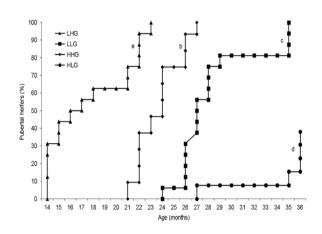
At that time, heifers were submitted to FTAI and a subgroup of heifers (n=39) were submitted to OPU/IVEP.

The results showed that heifers with genetics for sexual precocity produced a greater amount of quality embryos. This increase in embryo production was also observed in high nutrition heifers (Table 4). The FTAI pregnancy rate was higher in heifers with high nutrition (Table 5). Also, it was found that heifers with better nutrition and genetics for sexual precocity had greater deposition of subcutaneous fat, both in the rib (Graph 2 A) and in the croup (Graph 2 B). Thus, we conclude that both nutrition and genetics influence embryonic quality and carcass fat deposition. The data also showed that nutrition positively influenced the FTAI fertility of 14-month-old Nelore heifers (Catussi et al., 2022; unpublished data). These findings reinforce the importance of adequate nutrition and genetic selection for sexual precocity to obtain satisfactory results in reproductive programs in precocious Nellore heifers (14 months of age).



**Figure 2.** Subcutaneous fat thickness (mm) at the beginning of the FTAI protocol according to nutrition (IN vs. MN; Fig. 3A) and genetic groups (HG vs. LG; Fig. 3B). Subcutaneous rump fat thickness (RFAT) and subcutaneous backfat thickness (BFAT).

Ferraz et al. (2018) observed the effect of genetics and nutrition on the onset of puberty in Nellore heifers (Figure 2). The animals were distributed in the following treatments: LHG [superior nutrition (GMD 0.7kg/day) and superior genetics (DEP+ for age at first calving)], LLG [superior genetics and inferior nutrition (GMD 0.3kg/day)], HHG [inferior genetics and superior nutrition] and HLG [inferior genetics and nutrition]. There was an effect of nutrition and genetics on the onset of puberty (first ovulation). To reach 100% puberty under 24 months, it was necessary to combine superior nutrition and genetics.



**Figure 3.** Cumulative proportion (%) of Nellore heifers that reached puberty in each treatment. <sup>abcd</sup> Lines with different writings differ by P<0.05 (Ferraz, 2018).

#### UTERINE DEVELOPMENT AND SEXUAL PRECOCITY

Puberty can be defined as the period that occurs after the first ovulation, followed by a luteal phase of normal duration and with the possibility of conception (Atkins et al., 2013). Sexual maturity of the reproductive axis occurs gradually and is associated with changes in body composition and in the somatotropic axis, responsible for regulating the metabolic status and uterine development of the heifer.

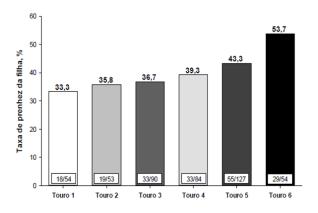
Day and Anderson (1998) report that the development of the reproductive tract can be classified as an infantile period followed by a developmental phase, which is associated with the release of GnRH and LH. Some authors have shown that the size and weight of the uterus, cervix and vagina increase rapidly after puberty (Desjardins and Hafs, 1969: Honaramooz et al., 2004), with prepubertal heifers having a smaller uterus when compared to pubertal heifers.

In this context, Freitas (2015) evaluated the reproductive tract score (RTE; classification described by Andersen et al., 1991) of heifers aged  $13.9\pm0.03$  months submitted to FTAI. In this study, there was no statistical difference (P=0.30) of the RTE on the pregnancy rate. However, in another study carried out by our group (Martins et al., 2017), the pregnancy rate of 14-month-old Nellore heifers submitted to FTAI was higher in heifers that had a uterus with a diameter greater than 1.5 cm (41, 1% for uterus 1.5cm vs. 17.8% for uterus < 1.5cm; P=0.0005).

Another important factor to consider for prepubertal heifers is cyclicity induction protocol. This treatment can be used as a strategy to increase the reproductive efficiency of prepubertal heifers. Cyclicity induction is performed with intravaginal P4 devices associated with estrogen administration (SÁ FILHO et al., 2015). Several studies indicate that this treatment increases ovulation induction (Claro et al., 2010; Rodrigues et al., 2014), potentially improving uterine development and reproductive efficiency in this category (Sales et al., 2009).

#### **GENETIC AND SEXUAL PRECOCITY**

Genetic selection is another factor that impacts the fertility of 14-month-old Nellore heifers (Bagley, 1993; Eler et al., 2002). Sexual precocity is a characteristic of high genetic heritability (h2=0.5), transferred from the bull to its offspring (SILVA et al., 2005). This concept was verified in the study by Freitas (2015), presented in figure 4. Also, in a project carried out in collaboration with the ANCP (National Association of Breeders and Researchers), Nelore heifers from genetically precocious bulls had a higher pregnancy rate, compared to bulls without genetic selection for sexual precocity [Early= 68% (44/65) and Not Early= 52% (69/133); P=0.03].



**Figure 4**. Conception rate at FTAI of 381 Nellore heifers aged 14 months 30 days, according to heifer paternity (Farm 1, breeding season 2014) (Freitas, 2015).

# SYNCHRONIZATION PROTOCOLS FOR PRECOCIOUS ZEBU HEIFERS

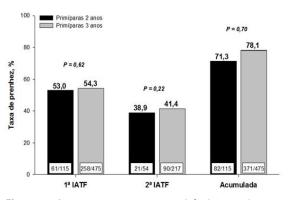
The synchronization protocols for FTAI most used in Bos indicus heifers present duration of permanence of the P4 device for 8 days (Sá Filho et al., 2010) or for 9 days (Sá Filho et al., 2009). However, 7-day protocols have been studied as an alternative for ovulation synchronization.

Factor et al. (2021) evaluated the duration of intravaginal progesterone device (7 or 9 days) and its effects on the FTAI pregnancy rate in 24.8-month-old Nelore heifers. Reducing the length of P4 device duration from 9 to 7 days promoted an increase in the FTAI conception rate [50.9% (111/218) vs. 59.1% (130/220); P = 0.04], which may be a tool to optimize the reproductive performance of this category. Felisbino et al. (2019) showed a high rate of early ovulation (30.3% of heifers ovulated between P4 device removal and FTAI) in 301 Nellore heifers synchronized with protocols with 8 days of P4 device permanence. Heifers that ovulated earlier had a larger FD diameter at the time of P4 device removal (11.1 vs. 9.6 mm, respectively) and lower pregnancy rates at FTAI (22.9% vs. 42.9%). Barbuio et al. (2020) found a reduction in the rate of early ovulation in Nellore heifers synchronized with protocols of 7 when compared to heifers synchronized with protocols of 8 days of permanence of the P4 device. The authors found a higher rate of early ovulation in heifers synchronized with 8 days (16.7%) when compared to heifers synchronized with 7 days (4.7%) of the P4 device permanence. These data support the possibility of improving the FTAI pregnancy rate of Nellore

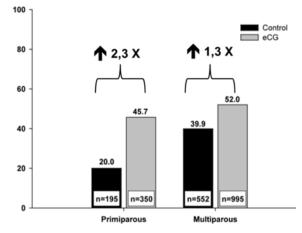
heifers synchronized with 7-day P4 device permanence protocols (Felisbino, 2021).

#### REPRODUCTIVE EFFICIENCY OF PRECOCIOUS PRIMIPAROUS

Another important analysis is the reproductive efficiency of heifers inseminated earlier when they will be primiparous at 2 years of age. In the study by Freitas (2015), early primiparous (2-year-old) and contemporary 3-year-old primiparous were submitted after calving to two consecutive FTAI (FTAI + resynchronization). The pregnancy rate of the first and second FTAI and the accumulated pregnancy rate were similar between these categories, demonstrating that under good management conditions there is no difference in reproductive efficiency between 2- and 3-year-old primiparous (Figure 5).



**Figure 5.** Conception rate at 1st and 2nd (resynchronization) FTAI of 2-year-old primiparous (precocious; n=115) compared to contemporary 3-year-old primiparous (n=475). The accumulated pregnancy rate (1st FTAI + Resynchronization) is also presented. Source: Freitas (2015).



**Figure 6.** Effect of treatment (Control or eCG) according to category (primiparous or multiparous) on the pregnancy rate at FTAI in Bos indicus cows.

These results demonstrate that, through adequate nutritional management combined with genetic selection for sexual precocity, it is possible to obtain also satisfactory reproductive efficiency in FTAI programs also in precocious primiparous. This strategy (heifers' early conception and primiparous reconception) also allows the reduction of the interval between generations, accelerating the genetic gain of the herd.

Furthermore, there is the possibility of increasing the reproductive efficiency of precocious primiparous with pharmacological treatments. In this context, equine chorionic gonadotropin (eCG) supports late-stage follicular development in synchronized heifers and cows. The use of eCG treatment, concomitant with the withdrawal of the progesterone device, improves pregnancy rates in this primiparous, as demonstrated by Sales et al. (2012), mainly in females with low body condition. These authors found that treatment with eCG has a greater impact on primiparous when compared to multiparous (Sales et al., 2016; Figure 6).

#### CONCLUSION

The technologies to reduce the age at first calving (i.e. selection of heifers with the ability to start their reproductive life early), is a basic requirement for the beef herd to become more productive. When the basic requirements (mainly nutrition and genetics) are met, the anticipation of the conception of heifers can be successfully established, improving the productivity and profitability of breeding livestock.

#### Conflicto de intereses

Los autores declaran no tener conflicto de intereses.

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