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Chapter

## Calf-Sex Influence in Bovine Milk Production

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#### Abstract

The main source of incomes in a dairy farm is milk sales, and any factor altering the production affects the farmers' income significantly. According to the Trivers-Willard hypothesis, if the cows' systems are generally good and offer competitive conditions, they produce more milk for bull calves. They also suggest that cows in a worse condition or of a genetically diverging strain invest more milk in heifer calves. The existence of a sex-bias in cows' milk production remains controversial even if it would open new insights on the economic impacts of using sex-sorted semen to enhance farm productivity. Sex-biased milk production in cows can vary, favoring one sex or the other and, sometimes, none. It seems to favor females in intensive production systems, while in other less intensive systems, this effect seems to disappear. This chapter intends to address available evidence on the sex-biased cows' milk production and discuss why further research forecasting this issue is needed, including other cattle populations and correlating the investment strategy with an animal welfare index. Besides, other factors, such as different housing and feedings, can impact the calf-sex milk production bias through pathways still to be understood.

**Keywords:** sex-biased milk production, secondary-sex effects, cattle, production system

#### 1. Introduction: Sex-bias in mammals mother resource allocation

Reproduction in mammalian females demands high energetic costs, driving the mobilization of fat deposits, in both gestation and lactation [1]. In evolutionary biology, numerous hypotheses defend a sex-biased allocation of these resources by the pregnant and nursing females, to maximize the reproductive success of their male and female offspring. Some of these theories support their reasoning in the local resource competition [2, 3], local resource enhancement [4], "advantaged daughters" [5], the "safe bet"/reproductive value [6, 7] and the sex-differentiated sources of mortality [8].

The most well-known and tested theory remains the Trivers-Willard hypothesis that predicts that: 1) females in good body condition will allocate her offspring sex ratio towards males; 2) and that mothers in good body condition will also invest more per son than per daughter if males exhibit greater variation in reproductive value when males exhibit greater variation in reproductive value [9] According to this hypothesis, female mammals are able to adjust the sex of their offspring based on their own condition as a form to maximize reproductive success in the next generation. This theory also states that the mother will adapt her milk production

to offspring gender, for example, by increasing milk production or changing its composition when she is nursing an offspring of the gender that has higher chances of producing future descendants. This strategy is particularly beneficial in species whose males compete for mating, like bovine, with dominant bulls leaving abundant offspring and weaker ones having no offspring at all. On the other side, this hypothesis also describes that investment in female offspring will be more profitable when the mothers are in poor condition because the chance of producing competitive male offspring is low. Well-nourished mothers invest more in male offspring, as strong sons will more likely leave more offspring, whereas even weaker daughters will produce more progeny than weak sons [10].

In agreement with this theory it has been shown that, in humans, the milk produced for males is more energy dense in well-nourished mothers [11], while mothers with low socioeconomic status, when nursing daughters produce milk with a higher fat content than when nursing sons [12]. However, evidence for systematic sex-biased favoring males has been equivocal [13–17]. Post-natal, sex-biased nursing care has been investigated as a possible reason for sex-biased milk production in several mammalians, including humans. Several studies reported evidence of sexbiased milk synthesis in different species but drawing definitive conclusions from these studies has been difficult for several reasons [11, 12, 18–30].

This chapter intents to discuss the evidences pro and against the existence of a sex-bias in cows' milk production, by stressing the putative effects of the calf gender in consecutive lactations while focusing in particular in dairy cows. Albeit non-consensual, its existence would open new insights on the economic impacts of using sex sorted semen to enhance farm productivity.

#### 2. Evolution of dairy milk production

In the last decades, industrial intensive milk production system uses the Holstein-Friesian breed, known as highest milk producing cow in the world. It is well documented that, with almost no exceptions, there has been a continuous increase in milk yield per cow. In all countries milk production and milk composition evolved over the years, due to a higher genetic merit and better management of the cows [31–32]. For example, from 2002 to 2013, in Denmark, all but two years showed a significant increase in the milk production compared to the previous year [33]. In São Miguel island, Azores, the same evolution was observed [34].

Also, in all countries, seasonal variations in milk production and composition were observed, both in intensive [35, 36] and pasture-based systems [37]. Previous studies have also proven that milk production varies with parity. It is generally observed a progressive increase in milk production in the first three to four parities and then a progressive decrease [38, 39].

#### 3. Bovine sex-biased milk production

The main source of income in a dairy farm is, by far, milk sale and any factor that can increase or decrease the production affect significantly the farmers income. Even though external factors like feeding, rearing and management are an important part of profitability, other factors, intrinsic to animals can have an important impact on profitability of a farm. The genetic merit [40] and sex of the calf are some of those factors. Beside the intrinsic difference in the commercial value of a female or a male offspring in a commercial farm, if the female milk production is indeed affected by the calf gender, then it could be a major factor for maximizing profits [41].

Calf-sex biased milk production is the capability of a cow to adapt milk production and composition to the sex of her offspring, a phenomenon well documented in diverse mammalian species [19]. The milk yield and the quality of milk produced are two important characteristics in dairy cow production and are also of great impact in beef production. Any favoring of one sex over the other in bovine offspring can lead to a great increase in the use of sex sorted semen, despite its lower conception rate [42]. In dairy cows, birth sex-ratio is biased, with more males being born, which suggests underlying mechanisms operating to favor more male offspring [43, 44].

Bull calves in dairy farms are mostly unwanted, due to their low value; in some countries, they are euthanized after birth, raising an ethical and social concern for the industry. On the other side, the used of sexed semen has higher costs and lower fertility. The fertility of sexed semen is estimated to be 8 to 17.9% lower in heifers compared to the conventional and not advisable to use in multiparous cows [42, 45, 46]. If a specific calf sex is associated with higher milk yield, this would have obvious consequences in the value and widespread use of sexed semen [46].

The growth rate of a suckling a male calf is higher than in females [47]. Therefore, it would be expected cows to have higher milk production or more energy dense milk when nursing a male. Despite differences in milk quantity or composition, cows do not show any sex biases in nursing behavior [29]. Since in most dairy farms, contrasting to most beef operations, calves are removed from the mother soon after calving, the pre and peri-natal mechanisms are the sole responsible for any observed milk-production sex-bias. Besides, cows are usually pregnant for most of the previous lactation [48], so the calf sex can potentially influence the previous lactation during its gestation or the lactation after their birth.

In *Bovidae*, data on the effect of calf sex in milk production are, to the least, inconsistent. Some studies reported an effect of calf sex on milk yield [1, 49–51], whereas other studies found no association [52]. One of the studies found that cows with a given genotype had higher milk yield in case of a male calf than a heifer calf [51]. In buffalos no effect between calf sex and milk production was reported [53].

In dairy cows in particular, studies addressing milk production sex-bias so far led to different results. While most studies described an advantage of female offspring, this effect was not observed for all the populations and a significant difference was not always observed [1, 33, 34, 40, 41, 54, 55]. Canadian and Iranian data for calf-sex bias in milk production found milk yield to be increased when a heifer was calved [41, 58]. However, a higher milk yield after calving a female offspring was only seen in the second lactation in New Zealand Holstein-Friesians [40], and only in the first lactation in French Holstein-Friesians [54] (**Table 1**).

However, Hinde et al. [1], with the largest study done so far on this topic, documented sex-biased milk production in US Holstein cattle. In his population, cows favor daughters, producing significantly more milk for daughters than for sons across lactation, suggesting that the effects of fetal sex can interact dynamically across parities. The sex of the fetus being gestated can enhance or diminish the production of milk during an established lactation. Moreover, the sex of the fetus gestated on the first parity has persistent consequences for milk synthesis on the subsequent parity. Contrastingly, Gillespie et al. [57] did not detect a significant effect of the sex of the calf being gestated on the mother milk production. Dallago et al. [55]. found only a calf-sex effect on the lactose and total solids, with an advantage to the females.

On a population of 1.49 million cows from the late 90's, primiparous cows giving birth to a female produced, on average, additional 142 kg (1.3% increase) of milk over a standardized 305-day lactation period compared with those calving a male [1]. The fetal sex on the first parity had also persistent effects on milk production

Country	Years analyzed	N (lactations)	Breed	Main results	Study (chronologically
USA	1995-1999	2,390,000	Holstein	Cows favor daughters, producing significantly more milk for daughters across lactation. Effects of fetal sex interact dynamically across parities, whereby the sex of the fetus being gestated can enhance or diminish the production of milk during an established lactation. Sex of the fetus gestated on the first parity has persistent consequences for milk synthesis on the subsequent parity. Specifically, gestation of a daughter on the first parity increases milk production by, 445 kg over the first two lactations	[1]
France	2000-2008	8,901,000	Montbéliarde and Holstein	The effect of the calf sex hardly affected milk production. A small effect in favor of males was observed in parity 2 and 3 and was similar across breeds. The estimated effect of the sex of the calf in gestation on the simultaneous lactation was also very small.	[53]
Iran	1992-2008	402,716	Holstein	Cows with female calves had higher milk and fat yield, persistency of milk and fat yield and longer lactation length, while cows that gave birth to male calves had shorter calving interval and longer productive life. Also, cows with female calves had higher milk yield per day of lactation in the first two parities, but there was no difference in milk yield per day of lactation for parities ≥3.	[40]
Denmark	till 2013	71,088	Holstein	Cows produced higher volumes of milk if they had a bull calf, with a significantly higher milk production of 0.28% in the first lactation period for cows giving birth to a bull calf. Such difference was even higher when cows gave birth to another bull calf, so having two bull calves resulted in a difference of 0.52% in milk production compared to any other combination of sex of the offspring.	[32]

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Country	Years analyzed	N (lactations)	Breed	Main results	Study (chronologically)
New Zealand	1995-2005	578,912	Holstein and Jersey	The lactation initiated by the birth of a female calf was associated with a 0.33–1.1% ( $p < 0.05$ ) higher milk yield. Female calf gender present carryover effects associated with higher milk yield in second lactations for Holstein Friesians (0.24%; $p = 0.01$ ) and third lactations for Jerseys (1.1%; $p = 0.01$ ).	[39]
UK	2013-2014	211,932	Holstein	Giving birth to a heifer calf conferred a 1% milk yield advantage in first lactation heifers, whilst giving birth to a bull calf conferred a 0.5% advantage in second lactation.	[46]
Azores, Portugal	2009-2017	45,712	Holstein	Whether calving a male or a female, cows roughly produced the same amount of milk and protein content. However, the content of fat was slightly higher when they calved females (P < 0.001).	[33]

 Table 1.

 Studies done so far to evaluate the effect of calf sex in Holstein cows' milk production.

during the second lactation. Calving a female on the first parity, increases milk production by 445 kg over the first two lactations, identifying a dramatic and sustained programming of mammary function by the offspring in utero. On the other side, cows calving a male son on their first parity produced less milk on their second lactation (P < 0.001), particularly if they also gestated a male calf on the second pregnancy (**Table 2**). According to the same study, the milk composition was similar whether the gestation produced a gestation of a son or daughter; the fat concentration was 3.61% after gestation of a daughter and 3.62% after gestation of a son; protein concentrations were the same (3.17%) [1].

Gillespie et al. [57] also showed that, in the UK, calving a heifer was associated with a 1% milk yield advantage in first lactation heifers, but calving a bull calf conferred a 0.5% advantage in second lactation. Heifer calves were also associated with a 0.66 kg reduction in saturated fatty acid content of milk in first lactation, even though there was no significant difference between genders in the second lactation. Interestingly, the effects of calf gender observed on both the yield and saturated fatty acid content were considered minor compared to the nutritional and genetic influences. Aspects that affect milk production, such as mastitis [58] or lameness [59], seem to have a deeper impact on milk production than calf gender.

An Iranian study, using 402,716 Holstein milk records from 1991 to 2008, report that cows calving a female offspring present a higher milk and fat yield and longer persistency of milk and fat yield, as well as a longer lactation length [41]. Cows calving a male offspring presented shorter calving interval and an overall longer reproductive life. The observed higher daily milk yield after calving a female in the first two parities was not maintained for the next parities [60]. However, a higher occurrence of dystocia in male calving was not taken into consideration and was most likely a factor for the higher milk production observed after calving a female calf [41]. In contrast, both a French [54] and a Danish [33] studies found a small increase in milk yield in both Holstein and Montbéliarde dams calving a male offspring. On the French study, the sex-bias favoring males effect reached 40 kg milk (0.5% of the mean), 0.6 kg fat, 0.6 kg protein. A small difference was also noticed for fat and protein contents (from 0.01 to 0.02%) in parity 2 and 3. Similarly, the estimated effect of the sex of the calf in gestation on the simultaneous lactation is very small [54].

Græsbøll et al. [33] also reported significantly higher milk productions (0.28%) in first lactating cows producing a bull calf. This difference was even higher when cows calved another bull calf, with a difference of 0.52% in milk production compared to any other possible combinations of offspring sex. The same study pointed that dams would favor a bull fetus by decreasing milk production during the second pregnancy if the calf born in the first parity was a heifer, which diluted the positive effect on milk production of calving a male in the first pregnancy. Being pregnant with a bull fetus may reduce milk production to possibly increase the energy spent

Differences	Calf gender combination at the first and second lactation				
Kg (%) —	Female-female vs. male-male	Female-male vs. male-male	Male-female vs. male-male		
at first lactation	24 (0.3)	7 (0.1)	13 (0.2)		
at second lactation	52 (0.6)	5 (0.1)	53 (0.6)		
Cumulative effect	76 (0.9)	12 (0.2)	66 (0.8)		

Table 2.

Effect of the calf gender combination at the first and second lactation (305d) according to Hinde et al. [1].

on the bull fetus. Also, cows seem to favor living bull offspring over unborn bull offspring, but unborn bull offspring over living heifer offspring [33].

The magnitude of sex bias milk production, when observed in other species, seems to be stronger among first parity females [11, 26, 27, 30, 59]. The effect of fetal sex may diminish to some extent in multiparous females due to the aggregate effects of sequential gestations of different fetal sexes on the mammary gland architecture. Alternatively, maternal investment tactics may change as a function of residual reproductive value [61] or targeted effort during critical developmental windows [62]. Interaction effects were observed between calf gender across the first three parities, with the lowest second parity milk yield observed when a cow gave birth to male calves in all three parities. First parity calf sex did not have a significant effect on the third lactation milk yield. Disparities between the effects for calf sex sequences that differed only by the calf gender in the first parity were not significantly different from each other [40].

In cows' populations were a daughter-biased milk production was observed, this may involve life-history tradeoffs for both cows and their daughters. High milk production in dairy cows has been associated with reduced fertility, health, and survival depending on environmental conditions [63]. It was also observed that cows gestated during lactation have moderately reduced survival and milk production in their own adulthood [48, 64].

Some of the differences found across different studies could be partly explained by differences in the datasets used; Hess et al. [40] used total lactational yield, calculated using the test interval method; Hinde et al. [1] and Barbat et al. [54] used the test day model rather than predicting 305 day milk yields; Graesboll et al. [33] adopted a farm-based approach using Wilmink curves to calculate 305 day milk yields and Gillespie et al. [47] used the Milkbot lactation model, that can be affected by environment and genetics [65]. Also, the use of sexed semen was not known in most of the studies and it can have a significant impact in the results obtained. Sexed semen is mainly used to breed heifers with higher genetic merit [54, 66] and this creates an obvious bias towards female calves. This can be aggravated by the fact that heifers inseminated with sexed semen tend to have lower fertility and become pregnant later, consequently calving in an older age, which is associated with a higher milk production [67].

The effect of the calf gander can further interact with other factors, like parity or seasonality, making it difficult to evaluate it in a precise way. It was observed that after the third calving, the mother milk production was independent of the calf gender. This observation might be related to larger pelvic dimensions of older cows and by consequence a lower incidence of dystocia [41].

A significant difference between the dairy industry in Azores [34] or New Zealand [40] compared with other populations is that both are primarily pasturebased. The production and calving in Azores are not, however, as seasonal as the one observed in New Zealand [34, 40]. In the non-seasonal pasture-based system no calf-sex bias in milk yield was observed, even though a slight increase in fat percentage was associated with the birth of a male calf [34].

In Denmark, the difference observed in milk production due to the sex of the offspring was generally smaller than the difference between farms. Other management related aspects are more important for the milk yield registered and the differences identified might be due to size of the offspring rather than the sex, but size and sex might also have separated effects [33]. So far, no relation was observed among mean somatic cells count and the sex of the calf born [41, 68, 69], even though this parameter is often associated with the cow body condition [70].

Modeling complex biological features, such as milk production, is challenging due to the number of inherent and environmental aspects that can influence them.

Also, the statistical model used for analysis may influence to a certain point the results and data interpretation. One explanation for the differences of the several studies on calf-sex biased milk production can be related to the models used. For example, in one of the studies, Holstein Friesian cows calving males in the first three parities had significantly lower first lactation milk yield than cows calving two males followed by a female in the first three parities, but this observation is biased if models do not include lactation length. Also, there are no reasonable biological reasons why to test the effect of the gender of the third calf on the first lactation yield. In fact, the observed effect of calf gender on milk yield is due to an association between calf gender and milk yield rather than calf gender triggering a difference in milk yield. The alleged effect of the third parity calf gender on the first lactation milk yield was not apparent when lactation length was included in the models [40]. In beef cows, studies with limited samples led to different sex-biased milk production, pointing to either favors a son [71], or a daughter [72], or not show any sex-biases [73]. A study in the red Chittagong cattle found no effect of the calf-sex in milk production [74].

#### 4. Pregnancy and lactation length

In New Zealand, with a seasonal calving system, the calf gender was reported to influence milk yield possibly through the increased gestation length of male calves [40]. In that study, the milk production tests were performed on the same date for all cows, so those calving a male would have their tests performed, on average, 2 days earlier. However, when the lactation length (reported longer in male calves) was included in the model, no effects existed of the calf gender over the increased production of milk [40]. At least part of the reported difference in milk production due to calf gender, was really due to methodological issues. The interval-centering method used provide a 10.8  $\pm$  4.0 L higher milk yield if herd tests are 2 days later in lactation. However, the observed calf sex variance is too large to be explained only by this difference in herd test dates. When lactation length shortens depending on calving date, as well as the herd tests occurring 2 days earlier, the difference in milk yield is 26.9  $\pm$  6.2 L. This difference is similar to the observed effect of calf gender on milk yield, further supporting that this effect is, at least partially, due to the different lactation length when male calves are born 2 days later [40].

It is difficult to establish any association between the calf gender and a presumed sex-biased milk production or a sex-biased pregnancy length, because of various existing confounder factors that may permeate such interaction. Mean pregnancy in length male calves is longer than in females, the difference also being affect by breed and parity [40, 75]. Also, primiparous cows tend to present shorter pregnancies than multiparous cows, the calves born lighter [76], albeit the risk for dystocia is also higher for first calving cows.

Recently, Atashi and Asaadi [77], using 252,798 lactations on 108,077 Holstein dairy cows in Iran showed that multiparous cows with longer gestations performed better in lactation than primiparous cows. This study also showed that multiparous cows with short gestation length had a lower yield at the beginning of lactation and higher raising and declining slopes of the lactation curve compared with cows presenting longer or average length of pregnancy.

The production system may also interfere with milk production performance of dairy cows. In seasonal breeding systems, late calving cows usually have a shorter lactation since the entire herd ceases lactation on the same day [40]. The lactation length is usually longer in non-seasonal systems because the lactation can continue until the milk yield of an individual cow drops below a point when it is

more economical to dry the cow. In these conditions a weaker negative correlation between gestation and lactation length is observed compared with seasonal systems where all the cows are dried of on a single day [40]. However, even in non-seasonal systems lactation length was observed to be approximately four days shorter following the birth of a male calf compared to a female calf across the first four lactations [41]. Chegini et al. [41] found that cows calving female offspring had more persistent lactations than those that calving male offspring, suggesting that the lactation curves are different.

Still, there is some controversy regarding the best methodology to apply when modeling the milk production (whether in milk yield or composition) to adequately account the effects of the gender of the calf. This is not an easy task, because it establishes a complex interaction with other parameters (e.g., pregnancy length, dystocia, and some cow related factors) that may act as confounding factors. Lactation length is one important factor affecting milk yield per lactation, leading to the need to introduce correction factors for lactation length in the models for milk production in cows. Lactation length in itself has a negative relationship with the annualized production of milk and milk solids [78]. Also, the milk yield and milk production curves change according to the lactation number, the persistency of the peak and lactational length, the cow genetics and the number or milking frequency, among other factors. Such aspects should also be considered in the lactation modeling studies. Models construct evaluate the lactation curves should be used that take all possible confounders into account simultaneously. Therefore, further investigation is necessary to confirm whether the shape of lactation curves differ based on calf gender and identify potential biological explanations for any such difference.

#### 5. Calving difficulty

Calving difficulty is higher with larger calves [79]. It is also known that there is a higher frequency of dystocia in male calves' birth [60, 74, 79]. Dystocia significantly reduces the whole lactation milk yield [40, 50, 54, 69, 75–77], besides increasing veterinary treatment costs [76], and reducing cow fertility. After dystocia there is a higher incidence of metritis [77], ketosis [80, 81], both associated with a decrease in milk production. Also, an easy calving presumably leads to a higher milk production because it is associated with reduced stress and pain during calving, consequently leading to a lower energy imbalance that can cause more metabolic disorders [41].

Male calves are typically larger than females, and pose a greater risk of dystocia [1, 79, 82]. However, Hinde et al. [1] reported that sex-biased milk synthesis remained when analysis was restricted to a subset of females without record of dystocia, and included information on individual cows across the first and second parity, favoring females.

A Danish study found different results. Farmer assisted calving were associated with a higher milk yield while cows with no farmer assistance or with veterinary assistance during the most recent calving produced less milk. This means that mildly to moderate calving difficulties improved milk yield, while no assistance or the need for veterinary assistance decreased subsequent milk production. In the same study the interaction between sex of offspring and difficulty of calving was found to be insignificant [33]. Still, it must be also considered that dystocia might go unnoticed, nevertheless affecting milk production, which could lead to misreading of the sex-bias towards higher production after female calving because of unidentified or unrecorded dystocia [40]. The effect of the different degrees of dystocia in milk production or for how long they persist remain unclear [83–85].

In UK Holstein-Friesian cows, moderate calving difficulties resulted in higher milk production. It is possible that some births not needing help and human supervision may experience real difficulties that go unnoticed and are wrongly registered as an easy calving, when they might have had some difficulties without the farmer's notice. Furthermore, it is likely that cows with highly valuated genetic material may be offered calving assistance from the farmer more often [67].

A reduction in milk production was observed between days in milk 10 and 90 after veterinary-assisted calving compared with non-assisted calving, leading to the conclusion that non-assisted cows presented a flatter lactation curve after peak yield [69]. One of the reasons is a reduced dry matter intake in the months postpartum [86].

In Jerseys the effects of calf gender in mothers milk production were not as pronounced as in Holstein-Friesians [40], which can point to a genetic selection of calf-sex biased milk production.

#### 6. Biological pathways of sex-biased milk production

Dairy calves are usually separated from their mothers right after or within hours of birth and artificially reared; therefore, the differences observed on milk production of the mother should relate to factors affecting the lactogenesis in pre- or peri-natal period [40]. The pathways through which fetal sex may influence milk production are not yet fully understood. Sex-biased milk production may reflect differential cellular capacity in the mammary gland, programmed via hormonal signals from the fetal-placental unit, or post-natal through sex-biased nursing behavior [87]. Several hypothetical mechanisms have been explored in an attempt to explain the mechanisms that may explain a sex-biased milk production in bovine, albeit with discrepant results.

One possible mechanism may relate to the translocation of fetal hormones to the cow mammary gland via the maternal circulation. The concentrations of sexual hormones differ between male and female fetuses and can potentially enhance or inhibit mammary milk synthesis, if they get access to maternal circulation. In the bovine species, fetal steroid hormones are present from the first trimester [88, 89]. The hormones produced by the bovine fetus can cross the placenta to the cow circulation and calf sex influences hormonal levels in the mother [76, 90–94]. Thereby, variations in the blood levels of the hormones involved in lactogenesis may influence milk, dependent on the sex of the calf born [40]. In humans, higher concentrations of circulating androgens during the second trimester were associated with a lower probability of sustaining breastfeeding to three months post-partum, but the effect of fetal sex and the milk production were not directly analyzed [95].

Also, it is possible that the sex of the first parity calf affects milk production for the duration of the productive life of a cow due to the differences in the level of the hormones that influence mammary development, as it has been reported in mice [96], since dairy cows are first bred before they are fully mature, usually with only 60% of their adult weight.

Xiang and colleagues [97] showed gender variations in the placenta weight in both *Bos taurus* and *Bos indicus* pregnancies; the placenta of the male fetus present heavier total placenta weight, better placenta efficiency heavier fetus weight than female fetus. These differences might explain and favor the fact that male calves are usually heavier than the female's.

Differences in the amount of placental lactogen produced between female and male fetus could differently prime the mammary gland of the cow [1]. It is accepted that prolactin and placental lactogens have roles in mammogenesis and lactogenesis but the mechanisms of action of those hormones act are still in discussion, and

the role of the calf gender is still unclear [98, 99]. Albeit the information available for bovine is scarce, in humans, differences were found in the levels of placental lactogen in the umbilical cord blood in female and male pregnancies [100]. It was also been shown that glucose-to-insulin ratios were lower in women bearing a female vs. those bearing a male fetus [101]. Both insulin and glucose are important modulators of milk production. The fetal Insulin-like peptide 3 (INSL3) are raised in maternal circulation during pregnancy in male-pregnant dairy cows and diminished in female-pregnant cows [102]. It was also demonstrated that the level of this hormone directly affects milk production [103, 104]. In cows, Insulin and IGF-I concentrations, important metabolic mediators of the energetic metabolism and body condition, are negatively associated with milk yield during the production phase of the lactation [105].

Hienddleder et al. [106] showed that total thyroxine concentrations were higher in male pregnancies, while triiodothyronine concentrations were unaffected by fetal gender. Contrastingly, free thyroxine concentrations were higher in female pregnancies of *Bos indicus* genetics, while in the *Bos taurus*, the values for that hormone tend to be higher in male pregnancies. No gender-associated differences were found regarding the Insulin-like growth factors in this study. The changes in the thyroid hormones' concentrations may contribute to a different pattern in gene expression at the mammary gland, due to their galactopoietic role that sets the mammary gland' s metabolic priority during lactation [107].

Exploring another route, Chew et al. [108] showed that larger calves are associated with higher milk production, maybe related to higher concentrations of estrogen and placental lactogens during gestation. Indirectly, this could be one of the reasons why, in some cases, male calves are associated with higher milk production, since male calves are usually heavier at birth [109]. However, a negative correlation between birthweight and milk production during gestation was also found, leading to the hypothesis that the competition for nutrient between the fetus in gestation and the milk production for the current one would drive a diminished milk production. Yet, it cannot be ruled out that a high milk production is in itself responsible for a smaller birthweight of the calf in gestation [110].

Women giving birth to daughters show upregulation of epithelial/lactocyte genes, which may be associated with increased milk yield [111]. Also, in dairy cattle a sex-biased in nitrogen and energy metabolism during the transition period was observed [112]. Higher odds exist for a male birth in cows that lose less body condition after calving [113, 114]. The depth of the Negative Energetic Balance (NEB) experienced by these cows may affect the sex-biased production of milk to favor one sex or the other. The usually higher NEB that cows go through in more intensive systems may account for the results obtained under highly intensive conditions compared to the ones obtained under less stressful management. Roche et al. [113] showed that a higher loss of body condition score by the cow was associated with a higher rate of born females. Higher milk producer cows usually lose more body condition score and have a higher rate of female calves' gestation [114]. This might be the reason why it seems that the birth of a female is positive to milk production; however, the relationship between these factors might be the inverse, with higher producers having a higher rate of female calves [34].

Cow's milk production increases with the weight of the calf born [115], and male calves mean weight at birth is higher [82]. This difference in calf-sex birth weight can lead to the idea that the milk production is related to sex, when in fact it only reflects the birth weight [40]. Chew et al. [108] found no calf-sex bias in milk production when birth weight was included in the model.

The sex of the calf whose birth initiates lactation can influence the milk production in the subsequent lactation because of the hormonal influences on the mammary gland development or due to the calf sex effects on pregnancy length. Also, fetal sex can influence lactation production during pregnancy because cows become pregnant at peak lactation [109].

In the *Cervus elaphus* species, the red reindeer, dominant females give birth to a higher proportion of males than their subordinates. It is known that these dominant hinds produce higher levels of progesterone in the early days of pregnancy, and male blastocysts secrete interferon-tau earlier than females, so the hypothesis is that maternal recognition of pregnancy in dominant hinds is therefore more likely to be successful if the blastocyst is male [116]. Factors such as this at the time of maternal recognition of pregnancy in cattle could also affect calf sex, but this has not been studied yet.

Holstein heifers in the USA, even after administration of bST (bovine somatotropin) still produced significantly higher milk yield if they calve a female offspring, but sex-biased milk synthesis was not observed in parities two through five [1]. Even though hormones can cause sex-biased milk production, other factors such as birth, weight, lactation length and dystocia probably have a higher impact [40].

#### 7. The use of sexed semen

Sexed semen produces 90% of offspring of the desired sex, but the fertility is reduced in between 75 to 80% compared with conventional frozen semen [117], because the sorting process produces a higher level of damaged to the spermatozoa [118]. Usually, sexed semen is applied more frequently in heifers, to profit from their higher fertility. Also, the heifers selected to be inseminated with sexed semen are usually the ones with higher genetic merit, so they are the ones producing the replacement animals [66].

The use of sorted semen in dairy industry screws the gender ratios into the female sex, seeking the production of future genetically superior replacement animals. Under the sex-biased milk production framework, and according to some studies [1, 41, 57], it would be expected to observe an increase in milk yield in cows that calved a female in their first and eventually in the second parity. This effect would overcome any negative effects exerted by the calving of a larger male fetus (increasing the stress over nutrients partitioning between the fetus and the mother during pregnancy, and increasing the risk for dystocia) and variations in pregnancy length. On the other hand, the sorted semen being applied more often in heifers or primiparous cows, the former tending to present shorter pregnancy lengths [76], may also influence the results if the type of semen used does not enter in the model used. Attention should be paid when analyzing data from most studies, because usually the type of semen used in artificial insemination is not considered as a variable in the statistical model, which could affect the results.

After investigating the effect of sex-bias in milk production, using simulated data and considering different intensities of sexed semen in three different scenarios, two studies concluded that including sex-bias could increase profitability between  $\in$ 4.0 and  $\in$ 9.9 per cow per year [58, 119] (**Table 3**). On the other hand, it was also concluded that any increase in milk yield from cows calving a female calf was insufficient to warrant the use of sexed semen. The real influence of sex-biased milk production using sexed semen must be further studied before recommendations can be made into its economic impact [40]. Also, two different studies concluded that, even though there might be an effect of calf gender on a cows' milk production, the impact was not large enough to influence profit [54] or encourage the use of sexed semen [56].

Differences in milk yield per cow/year	Without sex bias	With sex bias	Simulation scenario	
Milk yield (kg of ECM)	36	48	Sorted semen used in 30% of heifers and	
Net return (€)	3.0	7.0	30% of cows	
Milk yield (kg of ECM)	66	99	Sorted semen used in 100% of heifers and 50% of cows	
Net return (€)	3.1	13.0		

Table 3.

Effects of the use of sex sorted semen on milk yield per cow/year considering two different simulation scenarios [109].

#### 8. Conclusions

Whether or not a sex biased milk production in dairy cows exists, this bias can vary, favoring one sex or the other and, sometimes, none. It seems to favor females in intensive production systems, while in other less intensive systems this was not observed.

The conflictual results obtained in different studies considering the cow may influence the sex of offspring suggest that the systems were cows are generally in good and competitive condition produce more milk for bull calves. They also seem to indicate that cows in a worse condition, or of a genetically diverging strain, apparently invest more milk in heifer calves. Up to now, conflicting reports have been presented to the scientific society, but differences among the models used make difficult to establish a clear relation between the gender of the offspring and the productivity of the cow. The different results observed are probably due to differences in the methodological approach, and the different influencer parameters used to calculate a lactation milk production, and in possible confounding factors that may not be completely identified. Also, other factors, such as different housing and feedings can have impact in calf-sex milk production bias in pathways still to be understood.

To further explore this theory, additional research is needed that includes other cattle populations and correlating the investment strategy with an animal welfare index. If the calf sex effect in milk production is present in a population, selection of bull mothers and progeny tested bulls may be biased due to the offspring sex, increasing the genetic progress towards more profitable cows, if this calf-gender bias is accounted for in breeding value estimation.

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#### **Conflict of interest**

The authors declare no conflict of interest.

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